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PARTS I–II] *SECTION B* [VOL. 24

RESISTANCE TO SUBMERGENCE IN WATER OF THE LARVÆ OF *PRODENIA LITURA* FAB.

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Received on September 3, 1953
(Communicated by Dr. Uma Shankar Srivastava)

THE water resistance power of some of the lepidopterous larvæ and pupæ has been known for a long time. But no detailed records of the same are available, except those by Lefroy (1908) who made a few observations on the pupæ, and by Ripley (1923) who observed that a larva of *Feltia subgothica* (Noctuidæ), which accidentally fell into water and remained there for two days, regained its senses after sometime on being taken out. He later on conducted a few experiments and concluded that the resistance to submergence increased with the increase of the subterranean mode of life.

The following experiments were conducted to find out the capacity for submergence in water of the larvæ of *Prodenia litura*, and its effect on the later life of the insect.

Before dropping the larvæ in water, they were thoroughly cleaned with soft brush and water—as suggested by Ripley to remove soil or any other particles that might carry air underneath with it. Larvæ of the last instar were dropped in batches of forty each simultaneously into high-walled glass

dishes containing water nearly two inches high. At fixed intervals (as stated in the table), the five larvæ from each batch were taken out and again dried well by means of blotting-paper, being left over in the open air.

It was noted that when the larvæ were dropped into water, some of them floated and did not sink even when allowed to remain in water for as long as eight hours. On examination these were found to be in an advanced stage of pro-moulting period, as was evident from the fact that they moulted much earlier than others. This supports Ripley's conclusion that due to coming in of a layer of air within their skin, their bodies become light and hence they float.

The larvæ when dropped in water, first curled up, but within a minute or so they unwound themselves and began to wriggle about, very often passing out fæces at the same time. In some cases, the movement continued for more than ten minutes, others became motionless and dropped to the bottom much earlier.

In its preliminary stages the experiment was performed separately in distilled water and tap water. The difference between the two was nearly negligible, so in subsequent trials only tap water was used.

The larvæ which were dropped for a longer period showed annular swellings round the metathoracic and first abdominal segments with a dark colour.

The first sign of regaining consciousness was observed as slight movement of the antennæ which were extended out and then slowly drawn in. This movement was soon followed by those of the thoracic legs and prolegs respectively. After sometimes, the larvæ began to crawl a little, expelling a small quantity of water now and then. Normal activity was resumed after a few hours, the time varying with the period of submergence. Slowly the body swellings noted above in the thoracico-abdominal and anal regions were reduced and the larvæ appeared normal in all respects.

The larvæ drowned for eight hours regained consciousness in fifty minutes while those drowned for eight to twelve hours took much longer time. All the larvæ drowned upto twelve hours regained consciousness and afterwards continued feeding, and completed their development. Those between twelve to sixteen hours also regained their senses and only one larva out of a group of five died. Most of the larvæ taken out after sixteen hours regained consciousness allright but were unable to feed and died in a day or two.

As death takes a heavy toll when the larvæ are submerged for sixteen to twenty hours, this period may be regarded to be of vital importance and may be conveniently called the minimum time required for death by drowning or the maximum time of resistance to submergence.

Effect of Submergence of Prodenia litura *Larvæ in Water*

Batch No.	Duration of submergence		Time taken to regain sense				Result whether died or lived	Duration after which started feeding	
			First movement		Body movement				
	Hrs.	Mts.	Hr.	Mts.	Hrs.	Mts.		Hrs.	Mts.
1	2	..	..	20	..	30	All lived	2	..
2	4	..	..	25	..	40	do	2	10
3	6	..	..	35	1	..	do	2	25
4	8	..	..	50	1	30	do	2	50
5	10	..	1	20	2	..	do	3	20
6	12	..	2	..	2	50	One died after a day	3	45
7	14	..	2	40	4	..	2 died one after 2 hours and the other after 4 hours	4	57
8	16	..	3	5	4	30	1 died immediately	6	15
9	18	..	3	45	5	35	3 died. Those regained sense died later on the same day	8	30
10	20	..	5	..	7	30	4 died, only one regained sense and died after 3 hours		

So far as the effect of submergence on pupation is concerned, it is clear from the table that submergence upto ten hours has got very little effect on pupation as all larva pupated and the moths emerged as usual, although they took a little longer time than the controlled larvæ, which had pupated on the same day as those under experiment. Those submerged between twelve to sixteen hours also pupated but while the majority emerged, taking longer time than the controlled ones, some died during pupation. After sixteen hours emergence, the larvæ die before pupation.

The present study supports the observations of Ripley that death takes place due to suffocation by lack of oxygen and by mechanical injury due to accumulation of water in the system.

I wish to thank Prof. S. C. Verma, Reader, Zoology Department, University of Allahabad, under whose valuable guidance the work was carried out and to the Head of the Zoology Department, Allahabad University, for providing laboratory facilities.

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DORMANCY OF POTATO TUBERS AS AFFECTED BY METHYL NAPHTHOXY ACETATE AND METHYL NAPHTHALENE ACETATE

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Research Fellow of the National Institute of Sciences, India

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Received on May 6, 1954

Introduction

One of the most important causes for the restricted cultivation of potatoes, in India, appears to be the enormous loss sustained during storage of this crop in summer and monsoon months. In the conditions of storage such as one has in India, over 30% of potatoes are lost each year. Apart from these, such diseases as the virus, early and late blights, ring rot, mosaic and others, greatly destroy the crop in the field.

In India the potato storage period is a very long one, ranging from March to October, thereby covering the hottest months of the plains. Therefore the problem of checking the sprouting and storage of potato tubers in a healthy and firm condition, both for seed and edible purposes, becomes a major one. Due to the inadequate methods of storage of potatoes, the prices of seed potatoes at planting time goes beyond the means of an ordinary cultivator to the extent that they may shoot up to twenty times those prevailing soon after harvest.

Work was started in this department by me on the effect of hormones in prolonging the dormancy of potato tubers with a view to discover cheaper methods of storage which could be used, on a large scale, by our farmers.

Materials and Methods

Phulwa and Majestic, the dominant varieties of Uttar Pradesh, were selected, their sizes averaged roughly $1\frac{1}{2}''$ in diameter.

The two varieties were purchased from the Government Agricultural Farm, Kanpur.

N.B.—Although the entire experimental findings, included in this paper, were done during 1951, the data were shifted and written during January-March, 1954. The authoress wishes to take this opportunity of thanking the National Institute of Sciences, India, for the Fellowship which enabled her to expedite the writing up of the manuscript.

Hormones

The following hormones were used:—

1. Methyl naphthoxy acetate.
2. Methyl naphthalene acetate.

They were obtained from Messrs. Pal Chemicals, London.

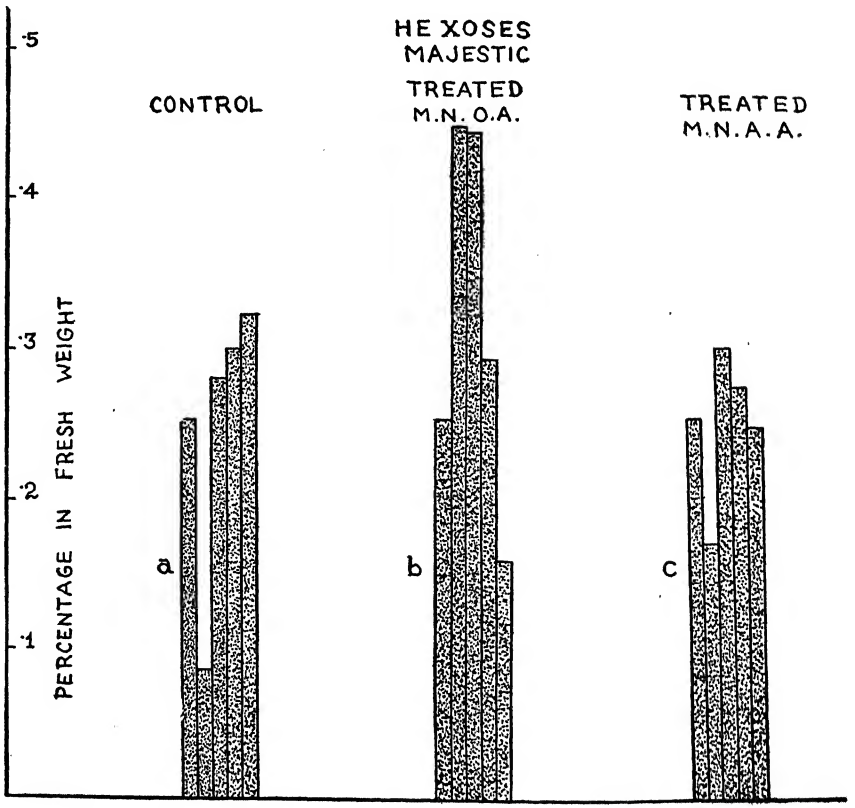

FIG. 1. Each column in the figure represents monthly readings from April to August to be read from left to right.

Method

Soil method of application was used throughout. This will be dealt in detail in the experimental procedure.

EXPERIMENTAL PROCEDURE

The varieties Phulwa and Majestic were harvested on 7th March 1951 and work on them was started on 21st March 1951.

*Treatments with MNOA and MNAA**

The hormones were applied at the rate of 50 mg., 25 mg., and 12½ mg. per 40 grams of soil for 40 potatoes. Ordinary garden soil was used for this purpose. It was previously oven dried, ground fine and passed through number 60 sieve mesh. Each of the above concentrations was thoroughly mixed with the soil. The various sets of potatoes were then rolled in the

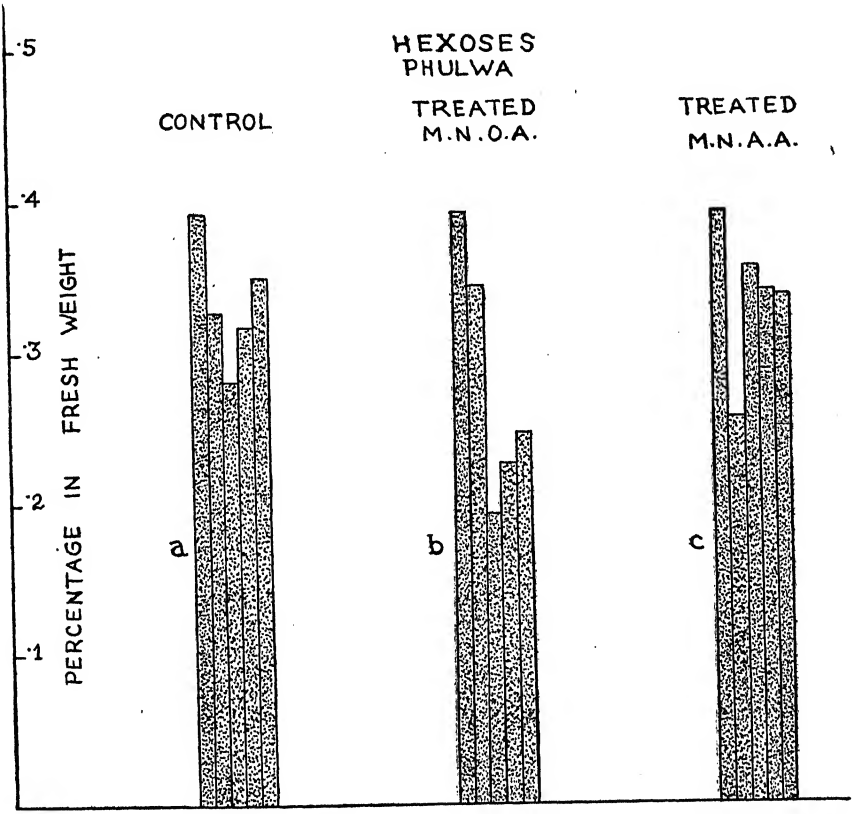

FIG. 2. Each column in the figure represents monthly readings from April to August to be read from left to right.

soil mixtures. With this fine dust adhering to the surface of the potatoes they were placed in wooden boxes. The control sets were similarly treated with the pure soil.

The treated and control sets were stored in uniform sized boxes covered with netted lids, and kept at room temperature (30° C. to 46° C.). Each of the above treatments was duplicated.

* The abbreviations used for the two hormones are: MNOA for methyl naphthoxy acetate and MNAA for methyl naphthalene acetate.

The storage period was from 21st March to 26th September 1951. At the end of the storage period, 6 tubers were picked under random sampling from each set, desprouted, and their sprout weights recorded.

Chemical studies

The sets treated with 50 mg. per 40 tubers of both hormones were selected for chemical analysis. The treated and control tubers were analysed at

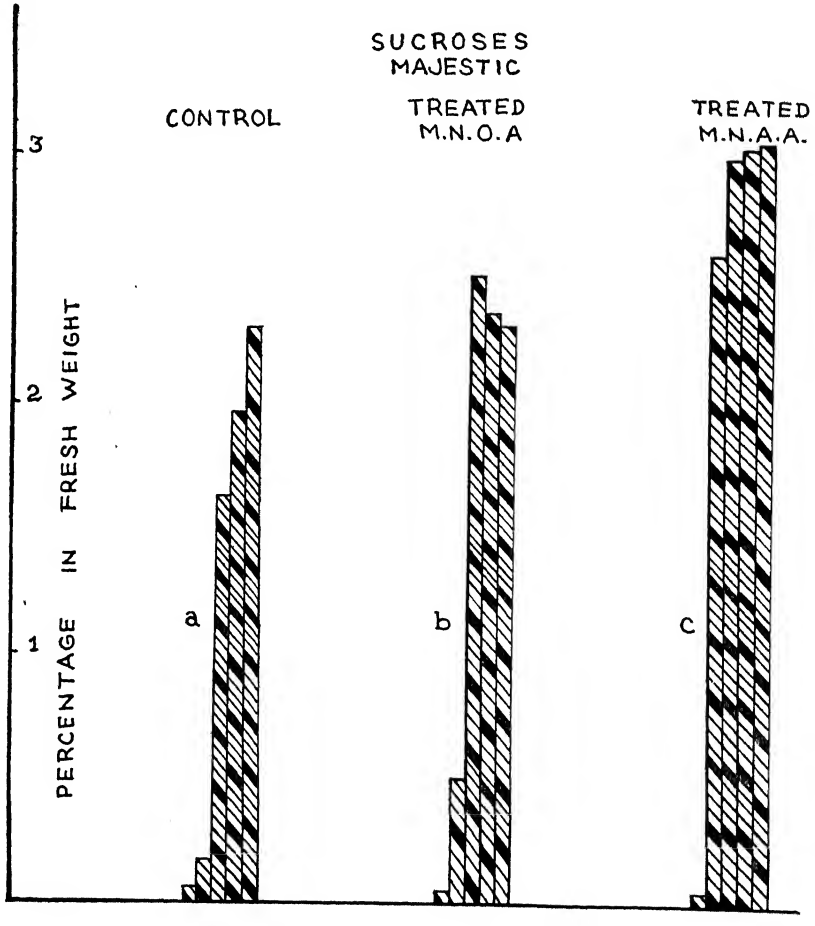

FIG. 3. Each column in the figure represents monthly readings from April to August to be read from left to right.

intervals of one month for estimations of total and soluble nitrogen, sugars, oxidases and dry weight.

Total and soluble nitrogen estimations were done by Kjeldahl's method.

The sugar estimations were undertaken by Somoegyi's method with minor modifications. The following sugars were estimated: (*a*) monosaccharides, (*b*) disaccharides and (*c*) total sugars.

Oxidases

The estimation of oxidases was done by Rohmann-Spitzer reaction method.

The chemical analysis were undertaken from April to August.

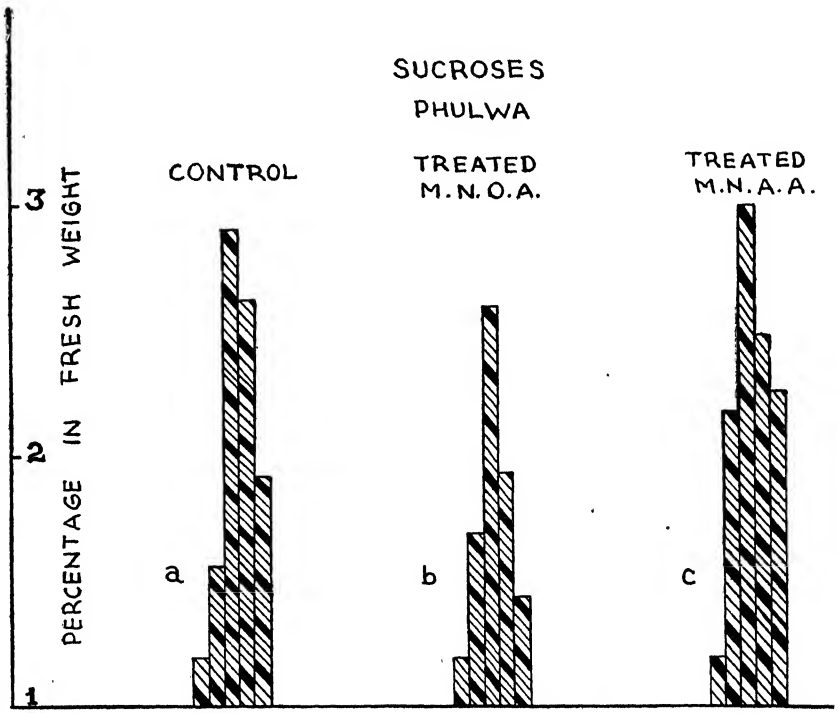

FIG. 4. Each column in the figure represents monthly readings from April to August to be read from left to right.

EXPERIMENTAL FINDINGS

MNOA and MNAA treatments on Majestic

The experimental findings were based on the fresh sprout weights of six tubers, picked under random sampling from each set, and on the chemical analysis.

Tables I and II show treatments of Majestic and Phulwa with MNOA and MNAA respectively.

A study of Table I shows that treatments with 50 mg. MNAA and MNOA were most effective in checking the growth of buds. Treatments with 25 mg. of both hormones were effective in inhibiting sprouting but not to the extent of the 50 mg. treatments. The least inhibition of sprouting was observed with 12½ mg. of both the hormones. The inhibition was slightly more in the case of MNAA treatments. When the two treatments are compared with the control, there is not much difference in their fresh sprout weights.

TABLE I

Variety Majestic: Treatments with Methyl Naphthaleneacetate and Methyl Naphthoxyacetate

Treatments with MNAA				Treatments with MNOA			
Conc. in mg. 1	†Fresh sprout weight in gm. 2	Average sprout weight of the three treatments 3	Rottage in number 4	Conc. in mg. 5	†Fresh sprout weight in mg. 6	Average sprout weight of the three treatments 7	Rottage in number
50 ..	·8340		10	50 ..	1·6430		6
25 ..	1·9684	1·9494	8	25 ..	2·8750	2·8530	9
12½ ..	3·0460		13	12½ ..	4·0410		13
Control	7·3413		15	Control	7·3413		16

†Fresh sprout weight of six tubers under random sampling in grams.

TABLE II

Variety Phulwa : Treatments with Methyl Naphthaleneacetate and Methyl Naphthoxyacetate

Treatments with MNAA				Treatments with MNOA			
Conc. in mg. 1	‡Fresh sprout weight in gm. 2	Average sprout weight of the treatments 3	Rottage in number 4	Conc. in mg. 5	‡Fresh sprout weight in gm. 6	Average sprout weight of the three treatments 7	Rottage in number 4
50 ..	·510		10	50 ..	1·3680		11
25 ..	·9431	1·666	9	25 ..	2·9486	2·7875	10
12½ ..	3·5460		14	12½ ..	4·0460		12
Control	5·1460		12	Control	5·1460		14

‡ Fresh sprout weight of six tubers under random sampling in grams.

MNAA and MNOA treatments on Phulwa

In analysing Table II, more or less the same trend of effectiveness, as seen in the Majestic, is observed. Here again, the 50 mg. treatments of both the hormones showed the best results. The 25 mg. treatments were

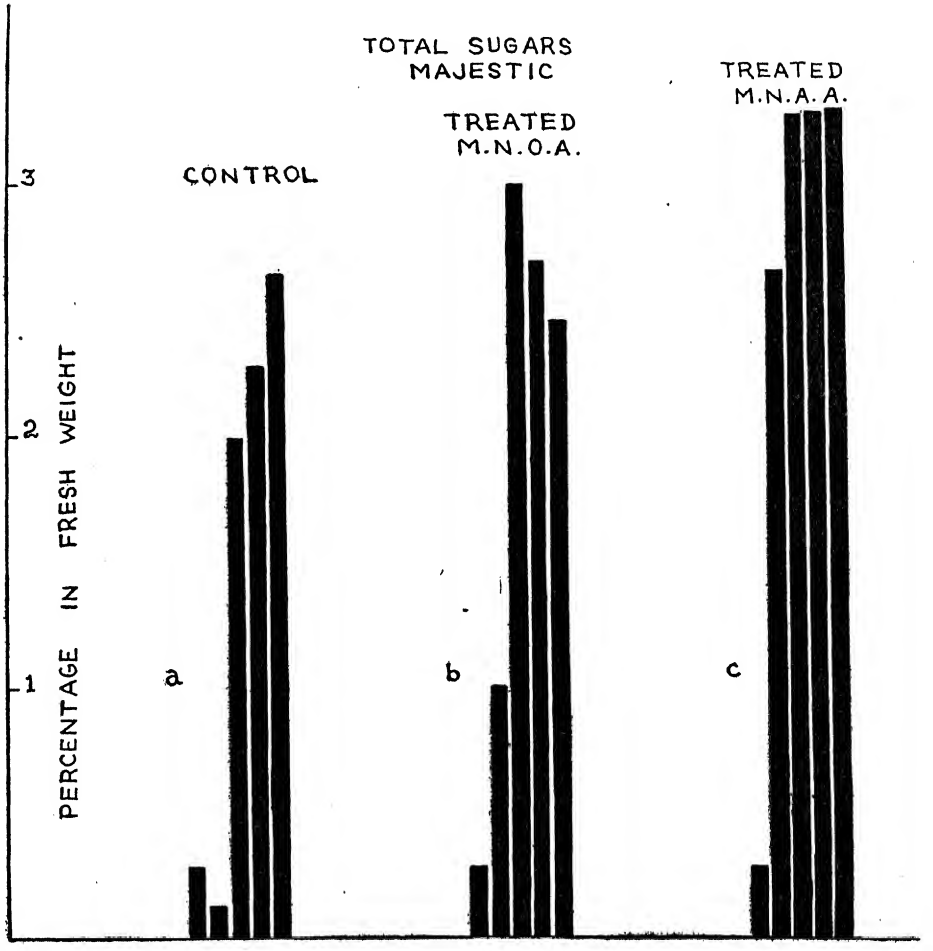

FIG. 5. Each column in the figure represents monthly readings from April to August to be read from left to right.

again not so effective as the 50 mg. treatments. Treatments with 12½ MNOA were least effective in checking the sprouting of tubers. Comparing with the control sets there is little difference in their sprout weights. It was however, observed that treatments with 12½ mg. MNAA were more effective than those with 12½ mg. MNOA.

Effect on rottage

Tables I and II show no appreciable effect of the two hormones, on the rottage of both Majestic and Phulwa. In general terms the rottage was more or less equal in all the sets, bein slightly more in the 12½ mg. MNAA and MNOA treated sets. The control sets showed slightly higher rottage than the treated sets.

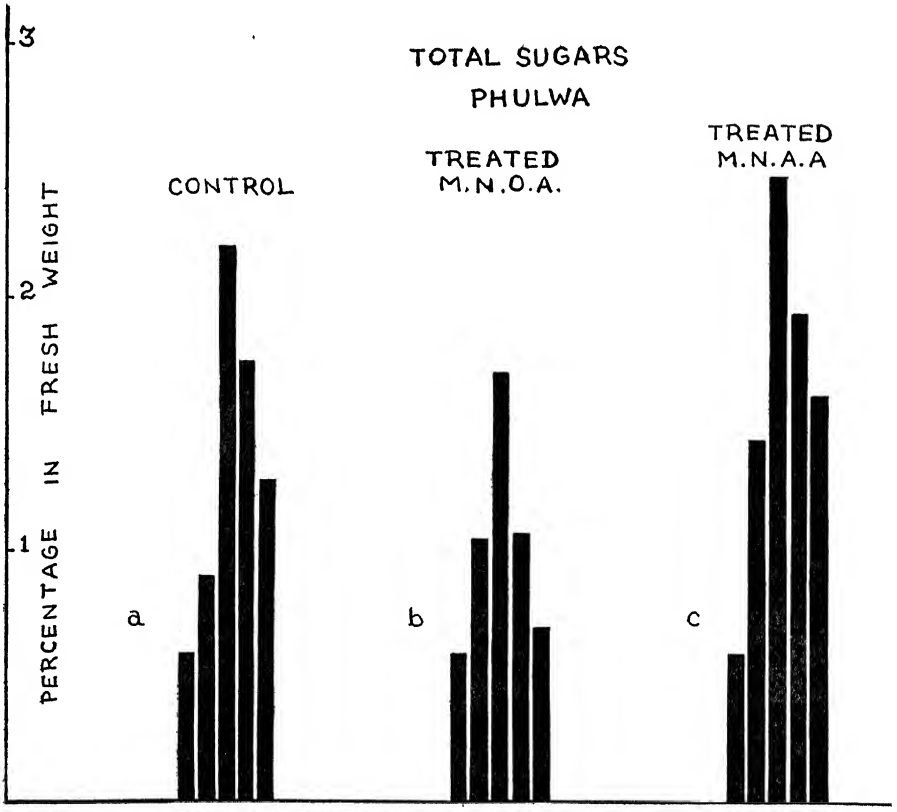

FIG. 6. Each column in the figure represents monthly readings from April to August to be read from left to right.

DISCUSSION

The experimental findings may be discussed as follows:—

1. The relative superiority of the two hormones in relation to the two varieties in prolonging the dormancy of potatoes.

2. Analysis of the chemical findings of the two treated varieties.

In the case of Majestic, treatments with 50 mg. of both hormones showed a superiority over other treatments. The next effective concentration was

25 mg. Tubers treated with 12½ mg. of both hormones showed only slight sprout inhibition.

Comparing columns 2 and 6 in Table I, one finds that all treatments, with the hormone MNAA were more effective than similar treatments with MNOA.

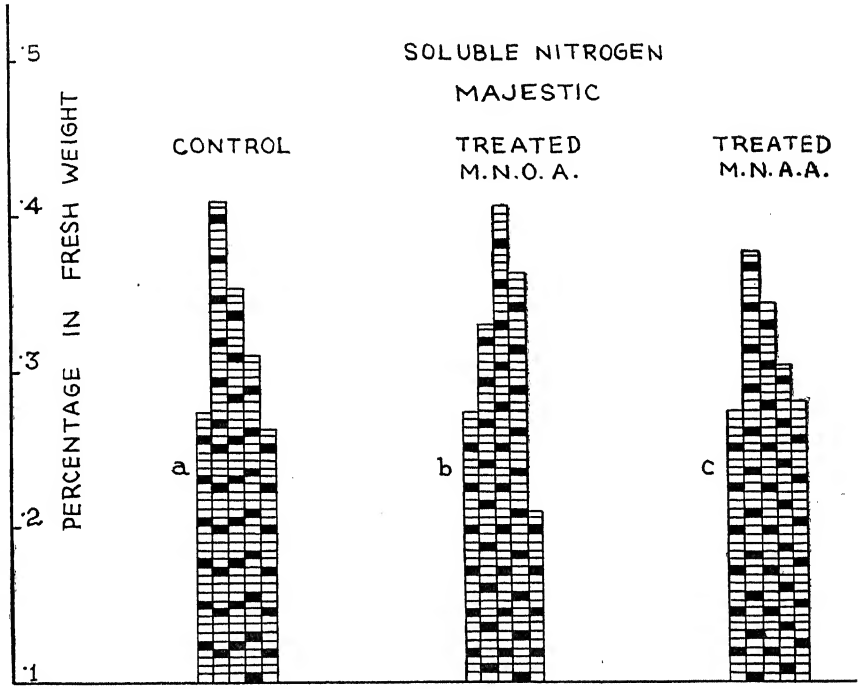

FIG. 7. Each column in the figure represents monthly readings from April to August to be read from left to right.

A study of Table II shows that the effectiveness of the three concentrations of both the hormones on Phulwa is very similar to those recorded for Majestic. 50 mg. treatments of both hormones gave the best results followed by 25 mg. treatments. Treatments with 12½ mg. MNOA and MNAA showed again only slight inhibition of sprouts when compared with the control set. Comparing columns 2 and 6 one again finds MNAA superior to MNOA.

Effectiveness of the hormones on Majestic and Phulwa

When considering the relative superiority of Majestic over Phulwa, in checking the growth of buds, one is able to conclude that treatments on

Majestic caused, a more or less, permanent dormancy of the tubers.§ An exception could be made with the $12\frac{1}{2}$ mg. treatments. Treatments on Phulwa were not so successful in checking the growth of buds.

Several hypothesis could be postulated in regard to the difference in the behaviour of Phulwa and Majestic. For instance the dormancy period of Majestic is about 3 months but sprouting usually takes place sometime between 2–12 weeks after harvest. In certain cases sprouting has been

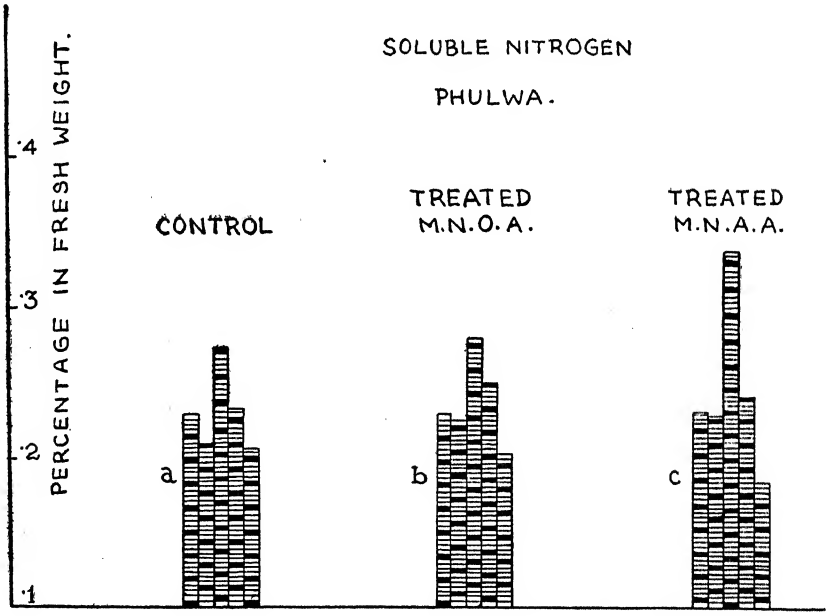

FIG. 8. Each column in the figure represents monthly readings from April to August to be read from left to right.

seen within a fortnight of harvesting. On the contrary the dormancy period of Phulwa is much longer, ranging from 3 to 5 months. When the experiments were started for prolonging the dormancy in March 1952, Majestic had started to sprout. On the other hand in Phulwa sprouting had not begun in the treated and control sets till July. It is possible that with the application of dormancy prolonging hormones the already sprouted buds of Majestic were killed. This did not happen in the case of Phulwa. The tubers here were in a dormant state when the hormones were applied. This explains the more or less permanent dormancy in the case of Majestic and not so in Phulwa.

§ The statement is based on the subsequent germination records, which showed a higher percentage for Phulwa.

Another possible explanation could be that the hormones affect only the bud regions and not the non-bud regions. Therefore, in the case of Phulwa, where the bud initials were in a very rudimentary state, the hormones were absorbed to a very small amount and consequently did not effect the

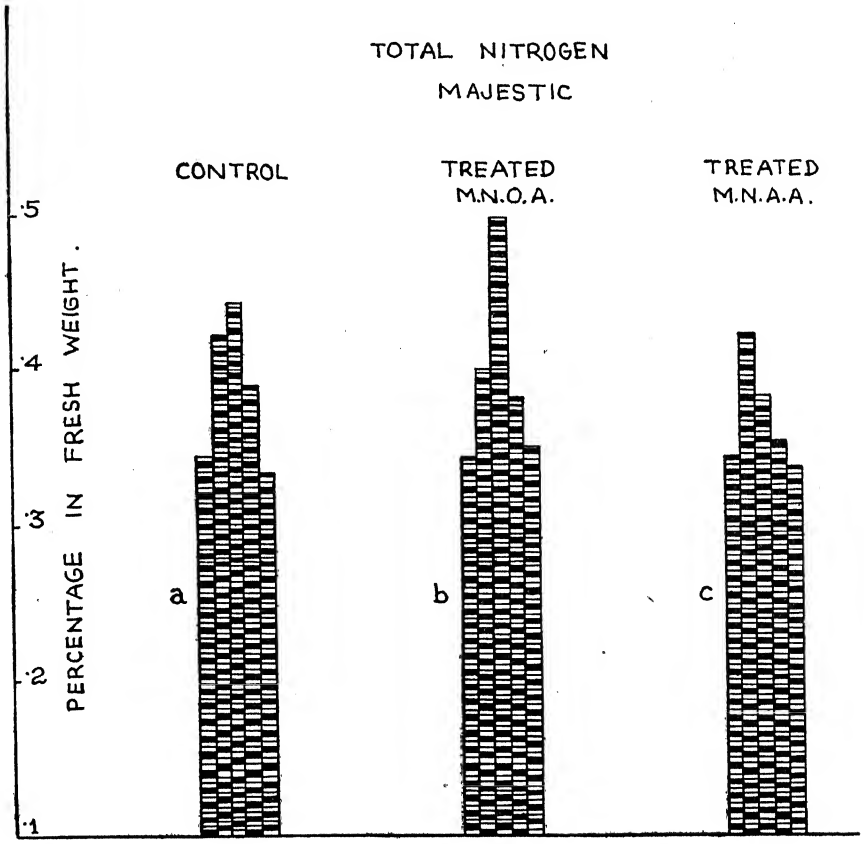

FIG. 9. Each column in the figure represents monthly readings from April to August to be read from left to right.

dormancy to such a marked degree as in Majestic. Here the buds had already grown to a sufficient size and thereby received a larger proportion of the hormone causing better inhibition of the growth of buds.

Analysis of the chemical findings of the two treated varieties

Fig. 1 indicates the amount of hexoses, during the months of April, May, June, July and August in Majestic and Fig. 2 in Phulwa.

Control: Majestic.—From the monthly readings of the hexoses in Fig. 1 (*a*) it is clear that this sugar, in the control set, steadily increases in the

successive months. The increase is far more pronounced in the case of sucrose [see Fig. 3 (*a*)]. This increase of sugars synchronizes with the bud development.

Phulwa.—Phulwa having a long dormancy period remains dormant during the months of April, May, June and July and therefore an increase in the respirable hexoses should not be envisaged. Fig. 2 (*a*) proves this, for the records from April to August show only minor fluctuations. Fig. 4 (*a*)

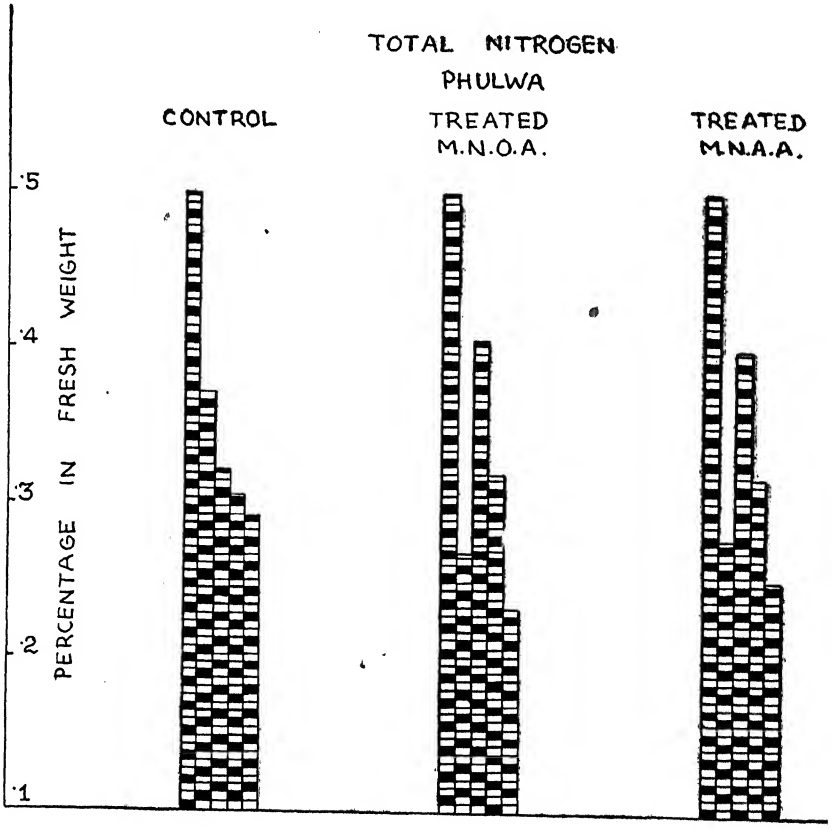

FIG. 10. Each column in the figure represents monthly readings from April to August to be read from left to right.

which shows the records of sucroses indicates a rise in June, thereafter there is a continuous fall. The hexose records a corresponding fall in this month. The temperature in June is the highest and therefore the sucrose ⇆ hexose equilibrium is likely to have altered to increase the sucrose with the corresponding decrease of the hexoses.

Treatments with MNOA and MNAA : Majestic.—Treatments with MNOA show rapid increase of hexoses in the months of May and June [see Fig. 1 (*b*)]. The hexoses then fall off during July and August.

The MNAA treatments [Fig. 1 (*c*)], on the contrary, did not increase the hexose to the extent that MNOA did. The sucrose records [see Fig. 3 (*b*) and (*c*)] however show a greater increase of the sugar in MNAA than in MNOA treated tubers.

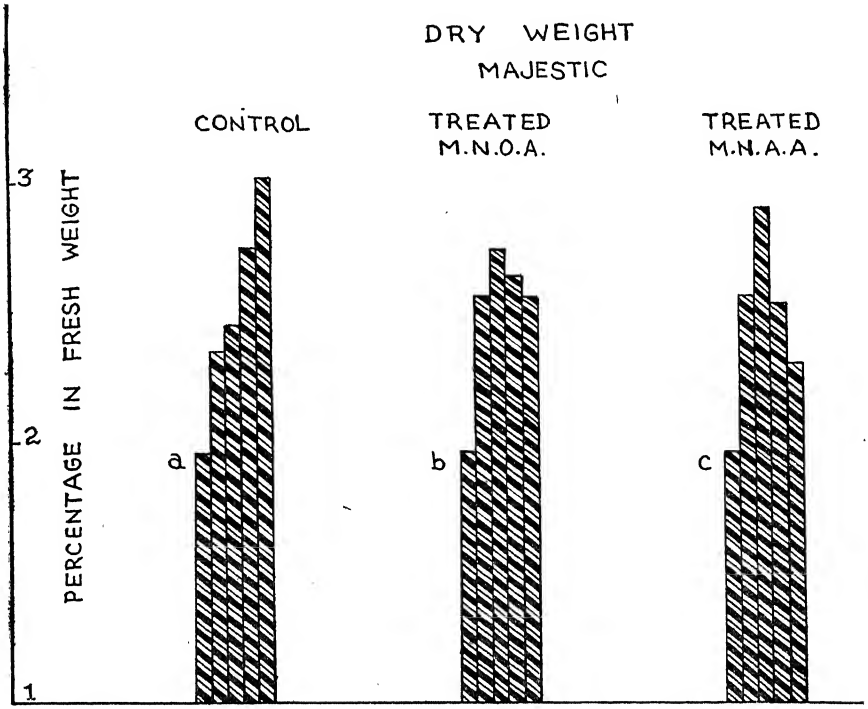

FIG. 11. Each column in the figure represents monthly readings from April to August to be read from left to right.

Sprouting being directly connected with the respirable sugars, it may be assumed that this increase of hexose would affect the bud growth far more than increase of the disaccharide sugars. Therefore, tubers treated with MNOA should give better sprouting than those treated with MNAA.

Phulwa.—As mentioned earlier Phulwa has a long dormancy period continuing practically upto the end of July. Therefore, the treatments with the various hormones for prolonging the dormancy would not show any difference with the control, during the months of April to July, so far as the hexose content goes. This is shown in Fig. 2 (*b*), (*c*).

Total Sugars: Majestic.—Fig. 5 shows that while in the control sets the sugars show a steady increase from April to August, there is a decline after a peak rise in June in the set treated with MNOA. The set treated with MNAA shows a rapid increase to a maximum in June and thereafter the value keeps steady.

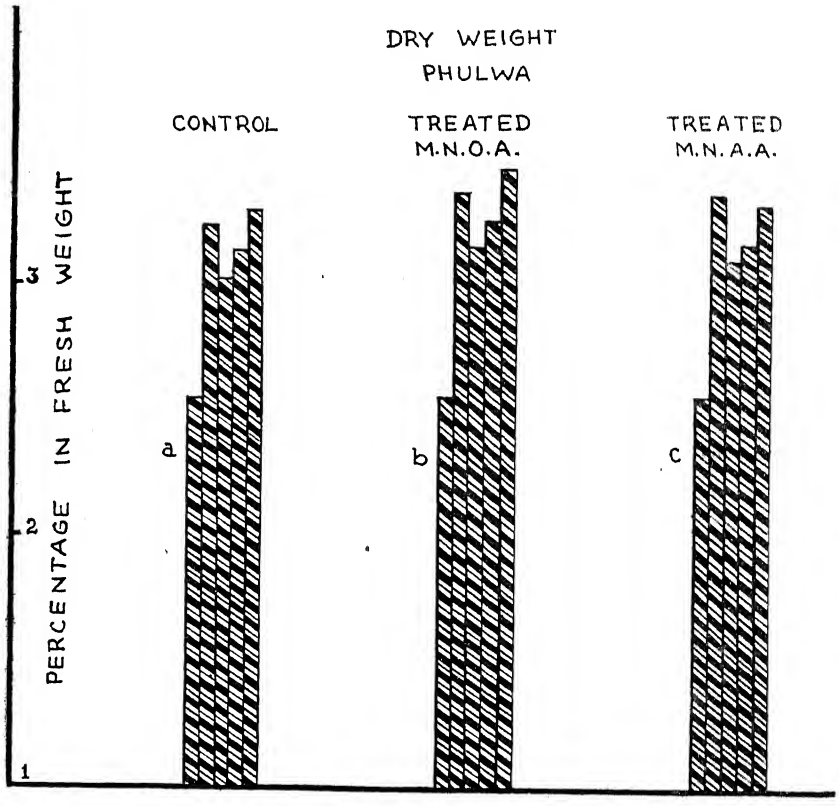

FIG. 12. Each column in the figure represents monthly readings from April to August to be read from left to right.

Phulwa.—A study of Fig. 6 shows that in all the three cases, *viz.*, control, MNOA and MNAA treated sets, there was an increase in total sugars upto June and then a subsequent gradual fall.

Oxidases.—A study of the oxidases of both Majestic and Phulwa unlike the sugars, does not give a clear picture of its relationship with sprouting.

Soluble Nitrogen.—From Figs. 7 and 8 it is apparent that, in the cases of both Majestic and Phulwa, the soluble nitrogen, in the control, MNOA

and MNAA treated sets, is more or less similar. However, in the Majestic it keeps a higher level.

Total Nitrogen.—Figs. 9 and 10 show hardly any significant differences in total Nitrogen in both Majestic and Phulwa. It may be assumed that the hormones did not appreciably effect the nitrogen metabolism of the tubers.

Dry weight : Majestic.—The dry weight of the potatoes recorded in Fig. 11 shows interesting results. Those of the control [Fig. 11 (*a*)] show a regular increase in their dry weight. On the basis of this the sprouting should really decrease as the moisture content decreases. Here, however, the reverse is true. Potato contains initially enough of moisture to enable the eyes to start germination after its initial dormancy period. The sprouting started in March and the germination went on right through till August. With the intense meristematic activity going on, there has been a continuous loss of moisture. This loss has, naturally, not been made good from external sources resulting in the proportionate increase of dry weight over moisture content. In the case of Majestic treated with MNOA, the action in prolonging the dormancy was not so marked as in MNAA and, therefore, the dry weight increases upto June, showing bud development till June and thereafter retardation. This is far more pronounced in the case of MNAA [see Fig. 11 (*b*)] for MNOA and [Fig. 11 (*c*)] for MNAA.

Phulwa.—In the case of Phulwa the bud development was far more restricted, even in the control, and one finds that though the dry weight increases from April to May, it keeps steady thereafter (see Fig. 12 *a*). This increase during April and May is undoubtedly due to hot dry climatic conditions. In the case of those treated with MNOA and MNAA there is no appreciable difference in dry weights (Figs. 12 *b* and *c*) as there was none in their respective sprouting.

Summary

In these experiments the varieties Majestic and Phulwa were treated with the hormones MNOA and MNAA for prolonging the dormancy of potatoes. The method adopted was soil treatment. Chemical analysis of the treated and control sets were also undertaken.

1. Of the two hormones used for prolonging the dormancy of Phulwa and Majestic MNAA was more effective than MNOA.

2. The variety Majestic, though with a shorter dormancy period, showed better dormancy prolonging behaviour than Phulwa.

3. Treatments with 50 mg. of both hormones showed the best sprout inhibition results. These were followed by 25 mg. sets. The sets treated with 12½ mg. showed the least inhibition of sprouts in both varieties when compared with the respective controls.

4. Sugar analysis in the case of Majestic show a distinct relationship between monosaccharides and bud growth. Greater amounts of monosaccharides synchronises with greater bud growth. Monosaccharides are poorer in tubers treated with MNAA than in MNOA, thus showing greater effectiveness of MNAA.

5. Dry weight is higher in the control. This is due to a relatively greater moisture loss as a result of bud growth.

6. The two hormones did not appreciably affect the nitrogen metabolism of the tubers.

7. Treatments with 12½ mg. MNOA, MNAA and control showed higher rottage of tubers than the other treatments used.

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REPRODUCTIVE ORGANS OF CERTAIN STORED-GRAIN BEETLES

III. Female Organs of *Sitophylus oryzae*, *Laria affinis* and *Rhizopertha dominica*

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INTRODUCTION

THE author has already described the male reproductive organs of *Sitophylus oryzæ* (Family Curculionidæ), *Laria affinis* (Family Bruchidæ) and *Rhizopertha dominica* (Family Bastrychidæ). In the present paper, he has dealt with the female reproductive organs of the same beetles.

OBSERVATIONS

(i) *Sitophylus oryzæ*

The Efferent System.—As usual, the ovaries are paired structures with each ovary consisting of only two small ovarioles, each measuring about 0·5 mm. long, and resembling in structure the ovarioles of *Rhizopertha*. In this beetle, however, the terminal filaments are absent and the ovarioles end anteriorly among the fat bodies. Consequently, the ovarioles are divergent and not convergent anteriorly. Each ovariole has only 2 or 3 developing ova and so these ovarioles differ from those of *S. granaria* which have 5 or 6 ova each. The pedicles of the ovarioles are much reduced and they open into the lateral oviduct of its side almost directly.

As in other cases, each lateral oviduct can be differentiated into two distinct regions—an anterior broad egg calyx with its wall composed of a layer of tall gland cells and a posterior, narrow lateral oviduct with its wall slightly infolded and composed of a layer of flat epithelial cells. The two parts are distinguished from each other by a slight constriction.

The two lateral oviducts unite with each other to form the common oviduct which runs backwards through the genital complex, formed by the common oviduct, the bursa and the spermathecal duct to open finally into the vagina. The wall of the oviduct is composed of cubical or flat cells surrounded by a thin layer of longitudinal muscles and then a thick layer of circular muscles outside, which is particularly well developed in the posterior part. The cells are lined internally by a thin layer of chitin.

The bursa copulatrix is a spacious pouch connected dorsally with the posterior end of the common oviduct and situated just dorsal to it in the genital complex. Its wall is composed of a layer of epithelial cells internally and circular muscles outside. Internally, it is lined by a fairly thick layer of chitin while externally, it is bound up with other structures in the genital complex.

The spermatheca is a small, strongly chitinous, U-shaped structure, measuring about 0·13 mm. long with its arms about 0·03 mm. in diameter. One of its two arms is slightly wider than the other. Its wall is composed of a thick layer of chitin with a broken layer of hypoderm inside. A few strong muscle fibres extend between the two arms and it appears that their contraction causes the two arms to be drawn nearer, thereby squeezing the spermatic fluid filled in it into the spermathecal duct. A small spermathecal gland, about 0·07 mm. in diameter, lies in close association with the spermatheca. It is composed of thin-walled, long-necked, somewhat triangular gland cells which pour their secretion into a narrow duct which runs through the gland to open into the wider arm of the spermatheca. Its secretion possibly acts as a nutritive or preservative fluid for the spermatozoids brought and stored into the spermatheca. The spermathecal duct originates from the spermatheca and runs posteriorly entering the genital complex from its dorsal side somewhere in the middle part. It runs through this complex and finally opens into the vagina near its junction with the common oviduct. It is composed of a layer of broad cells with a thin chitinous rind within and peritoneum outside.

The vagina into which the common oviduct, the bursa and the spermatheca finally open is a comparatively small but wide chamber with a well-developed layer of chitin within. On account of its rather shallow nature, it is often designated as the genital atrium which seems erroneous as this is the only part of the genital passage behind the opening of the spermathecal duct and hence it must play an important part during copulation. As suggested by Snodgrass, the point of union between the true oviduct and the vagina is marked approximately by the opening of the spermatheca into the anterior end of the latter. The cellular wall of the vagina, as well as that of the bursa, is lined by well-developed musculature and finally all the structures in the genital complex are surrounded by a few layers of longitudinal muscles and peritoneum which keep the organs bound together.

The ovipositor.—A well-developed genital chamber is formed by the invagination of the last three segments and the ovipositor is situated in it. Each ovipositor is distinguishable into three distinct parts—the valvifer,

the coxite and the stylets. The valvifer is a broad chitinized plate situated anteriorly. To this the coxite, measuring about 0·6 mm. long, is articulated by a thin membrane behind. It is more or less conical in structure with its narrow end directed posteriorly and bears a large number of chitinous hairs. Posteriorly, it bears a small ill-developed stylus furnished with sensory hair.

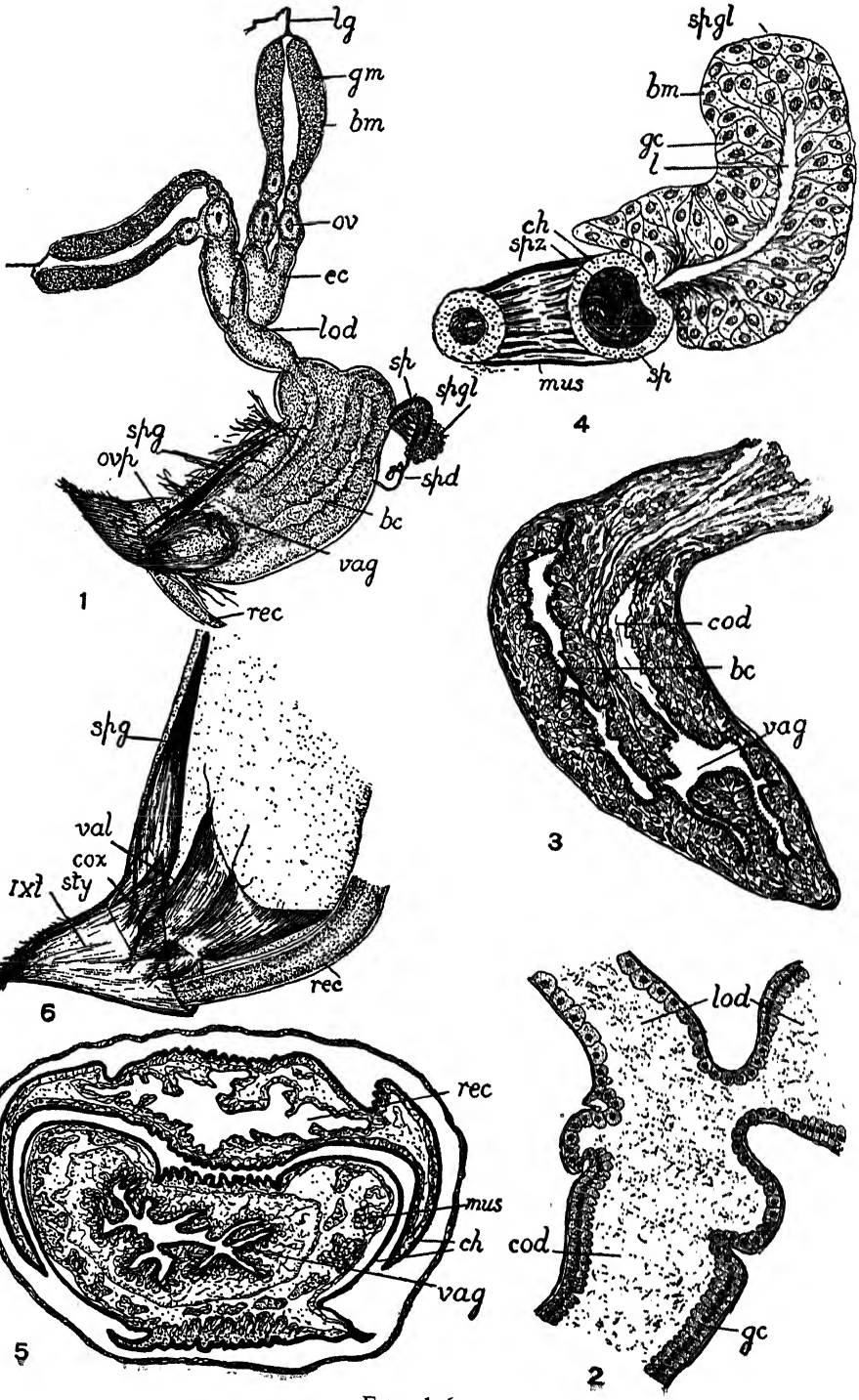
The spiculum gastrale is a dorso-ventrally flattened, chitinous bar, about 0·6 mm. long and situated on the ventral side of the genital complex with which it is intimately bound by muscle fibres. It is broad posteriorly and tapering anteriorly. Its posterior end remains in contact with the last sternite. The structure furnishes a surface of attachment for muscles controlling the movement of organs of copulation and oviposition.

(ii) *Rhizopertha dominica*

The Efferent System.—The ovaries are large paired structures, each consisting of eight acrotrophic ovarioles. A mature ovariole measures about 0·56 mm. long and extends from the second to the fourth abdominal segment. It contains only two ova towards its posterior side, the rest being filled by small oocytes, follicle cells and nurse cells. The anterior ends of the ovarioles are slightly swollen. A thin investing membrane covers the ovarioles; it is drawn out anteriorly to form the terminal filament. The filaments of the different ovarioles join to form the terminal ligaments. Behind the last ovary, a mass of cells forms a 'plug' to prevent the immature ova from passing down. There is no trace of muscle fibres in the ovarioles.

Each ovariole opens behind into the egg calyx (0·18 mm. in diameter) which opens by a comparatively narrow aperture into the lateral oviduct. The two lateral oviducts are small tubes which join each other to form the common oviduct. The walls of the egg calyx and the lateral oviducts are both composed of a single layer of gland cells arranged on an outer basement membrane which is thin in the region of egg calyx and thick in that of the lateral oviduct. When these ducts are dilated, these cells are flat and wide and when the ducts narrow down, the cells are tall. The cells produce a finely granular secretion which fills the cavity of the egg calyx and the oviduct and helps in the passage of ova downwards.

The common or median oviduct is a short, very thin-walled tube whose wall is also composed of a single layer of cells arranged on a thin intima and surrounded by peritoneum. Muscle fibres are absent both in the lateral and the common oviducts.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FIGS. 1–6

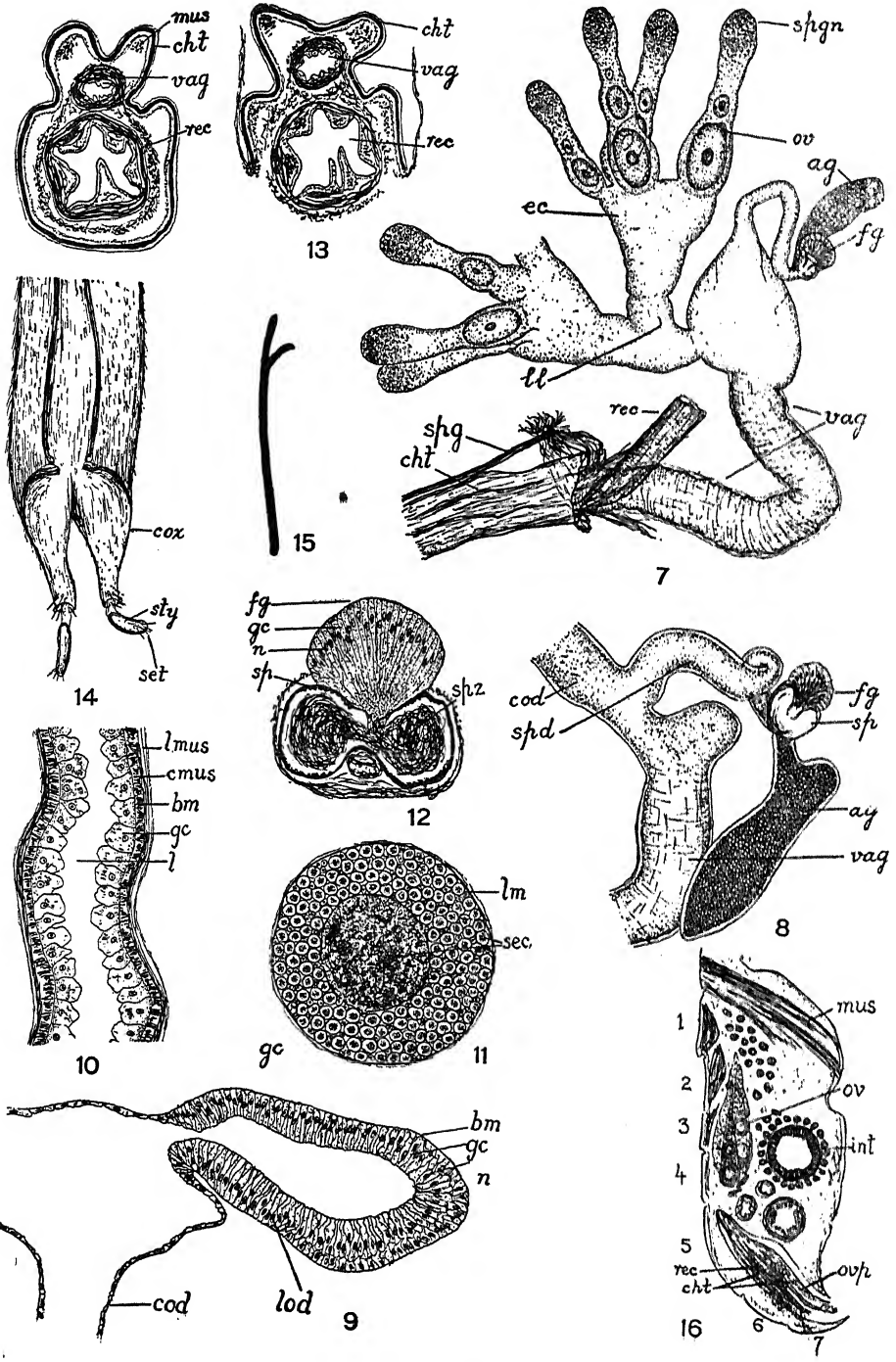
FIGS. 1–6. Female reproductive organs of *Sitophylus oryzae*. Fig. 1. Preparation of complete reproductive organs. Fig. 2. L.S. of lateral and common oviducts. Fig. 3. L.S. of the genital complex showing the relationship of the common oviduct, bursa and the vagina. Fig. 4. T.S. of spermatheca and spermathecal gland. Fig. 5. T.S. of the vagina. Fig. 6. The ovipositor and the spiculum gastrale.

ag, accessory gland; *bc*, bursa copulatrix; *bm*, basement membrane; *ch*, chitin; *cht*, chitinous tube; *cmus*, circular muscles; *cod*, common oviduct; *cox*, coxite; *ec*, egg calyx; *fg*, fan-shaped gland; *gc*, gland cell; *gm*, germarium; *l*, lumen; *lg*, ligament; *lm*, limiting membrane; *lmus*, longitudinal muscles; *lod*, lateral oviduct; *mus*, muscles; *n*, nucleus; *ov*, ovum; *ovp*, ovipositor; *rec*, rectum; *sec*, secretion; *set*, setae; *sp*, spermatheca; *spd*, spermathecal duct; *spg*, spiculum gastrale; *spgl*, spermathecal gland; *spgn*, spermatogonium; *spz*, spermatozoids; *sty*, stylus; *ter*, tergum; *vag*, vagina; *val*, valvifer.

The common oviduct leads behind directly into a long and broad tube, the vagina which is differentiated from the former by its different histological structure and by the opening of the spermathecal duct at the junction of the two. Compared to other beetles, here the vagina is very long, measuring about 0·9 mm. in length. It runs posteriorly for nearly one-third its length, reaching nearly the middle of the spiculum gastrale, when it abruptly turns ventrad and forward upto a little ahead of the proximal end of the spiculum. Here it again turns back, this time entering a long chitinous tube in the fifth segment. A strong band of muscle fibres binds the folded vagina with the anterior end of the spiculum rod and thus keeps it in position.

The wall of the vagina is thick and often infolded internally. It is composed of three layers. Its innermost layer consist of large, highly contractile gland cells with prominent nuclei. Outer to this is seen a thick layer of longitudinally arranged muscle fibres. A layer of peritoneum, continuous with that of the common oviduct, invests the vagina from outside.

The spermathecal duct, which has already been referred to, measures about 0·11 mm. long and 0·03 mm. in diameter and connects the spermatheca with the vagina. Its wall, composed of a single layer of cubical epithelial cells arranged on a firm basement membrane, is lined internally by a thin layer of cuticle. The spermatheca itself, which looks like a small strongly chitinous sphere, is really a double-walled cup with a narrow cavity between the two walls filled with spermatozoids received during copulation. In the depression of the cup is situated a fan-shaped spermathecal gland composed of radially arranged gland cells with their broad bases towards the periphery and the narrow ends drawn out to open into the depressed internal wall of the spermatheca. The nuclei of these cells are located towards their broad ends and the secretion which appears thick and granular on coagulation can be seen towards the openings. The secretion is thus poured directly into the spermatheca and, being of a thicker consistency, seems to serve as a nutritive fluid for the stored spermatozoids.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FIGS. 7–16

FIGS. 7–16. Female reproductive organs of *Rhizopertha dominica*. Fig. 7. Preparation of complete reproductive organs. Fig. 8. The common oviduct, spermatheca and spermathecal glands magnified to show their relationship. Fig. 9. L.S. of the lateral and common oviducts. Fig. 10. L.S. of the vagina. Fig. 11. T.S. of the accessory gland. Fig. 12. T.S. through the spermatheca and the fan-shaped gland. Fig. 13 *a* and *b*. T.S. through the different regions of the chitinous tube. Fig. 14. The ovipositor. Fig. 15. The spiculum gastrale. Fig. 16. L.V.S. of the abdomen showing the disposition of the organs.

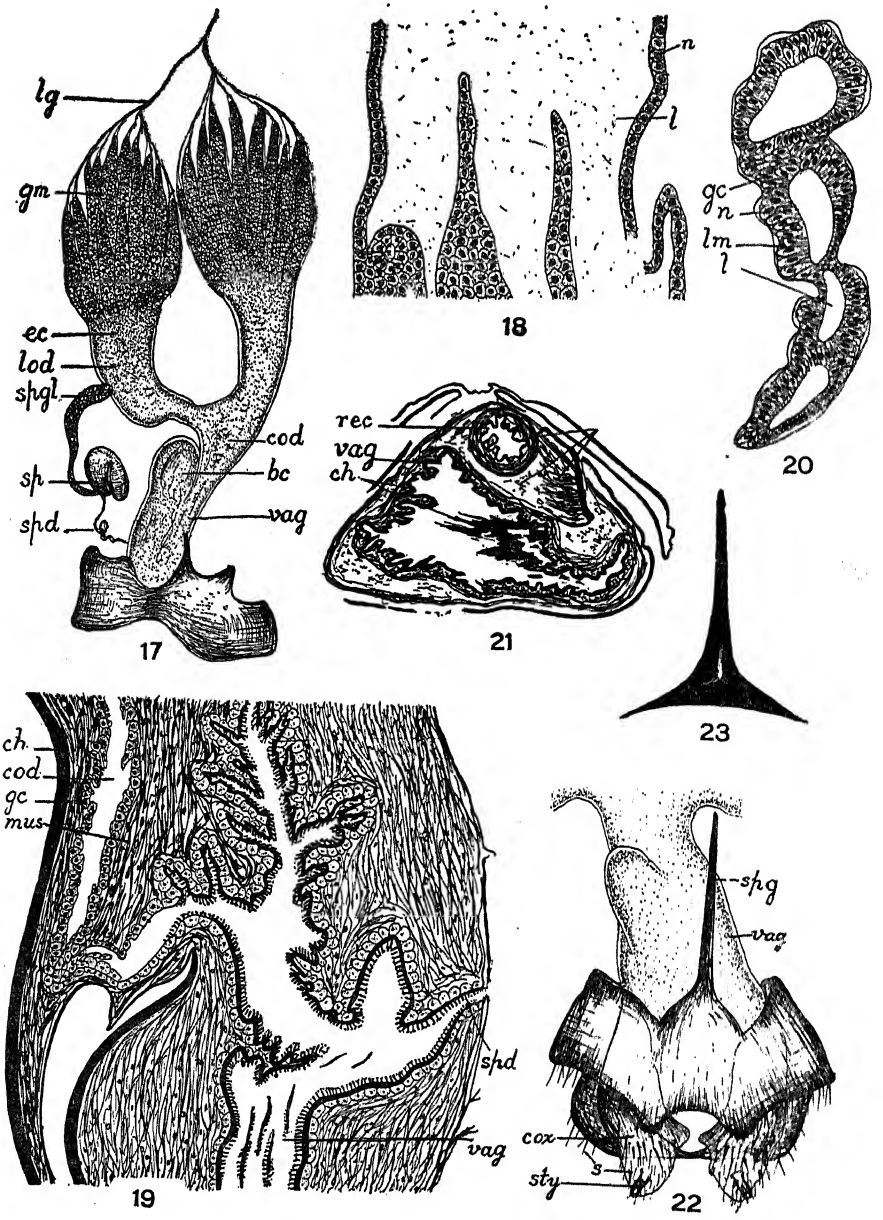
Lettering as in Figs. 1–6.

Another large, more or less banana-shaped accessory gland opens into the spermathecal duct near the opening of the latter into the vagina. It is about 0·42 mm. long, broader in the middle and tapering at the two ends, the diameter in the broadest region being 0·13 mm. In a cross-section it is found to be composed of a large number of fine chitinous tubules arranged longitudinally around a large central cavity. A few small cells with prominent nuclei are seen between the tubules and in some cells neck-like projections have been noted although their openings could not be ascertained. The innermost tubules communicate with the central cavity. Possibly, the interstitial cells pour their secretion into the tubules from which it eventually reaches the central cavity. The central cavity opens into the spermathecal duct. A fine limiting membrane invests the entire structure from outside.

The terminal part of the vagina, together with the rectum, is enclosed in the chitinous tube mentioned above. This tube is a double-walled structure divided by a pair of lateral notches into a dorsal and a ventral part. The dorsal part lodges the rectum and the ventral part the vagina. Of the two walls, the outer one is thinner and, in fact, represents the invaginated wall of the last body segment.

The ovipositor.—The last three segments of the body, with well-developed inter-segmental membranes, are invaginated and telescoped to form the genital chamber in which the ovipositor is lodged in the state of retraction. The ovipositor is a modified structure, the different parts of which are not distinguished from each other on account of their fusion and consequent disappearance of the intervening membranes. It is tubular in form and bears a pair of small, palpiform processes at its distal end. The processes are the genital palps or styli and bear minute sensory hair, serving the animal in the selection of the proper 'site' for oviposition.

The spiculum gastrale is a long, strongly chitinous rod, measuring 0·8 mm. in length and lying ventrally to the chitinous tube. Its distal end is bifid and the two arms which are attached to the sternites of the eighth segment are unequal and asymmetrical. The main rod is slightly curved. As stated before, strong muscles attached to its anterior end surround the



FIGS. 17–23. Female reproductive organs of *Laria affinis*. Fig. 17. Preparation of the complete reproductive organs. Fig. 18. L.V.S. of the lateral oviducts. Fig. 19. L.V.S. of the genital complex. Fig. 20. L.V.S. of the accessory gland. Fig. 21. T.S. of vagina. Fig. 22. Preparation of the ovipositor and the spiculum gastrale. Fig. 23. The spiculum gastrale.

Lettering as in Figs. 1–6.

folded vagina and keep it in position. Some other fibres attached to it run anteriorly to the spermatheca and the accessory gland while a few are connected to the fifth sternite. This arrangement obviously plays a very prominent part during copulation and oviposition when the spiculum acts as the lever or support for the movement of the various organs. The simultaneous contraction of the posterior and the anterior muscles will bring about pushing of the long vagina into the chitinous tube as well as the protrusion of the latter outside the genital chamber, thus enabling the ovipositor to come in contact with the surface on which eggs are to be laid and facilitating their passage outside.

(iii) *Laria affinis*

The Efferent System.—Unlike the beetles described above and other bruchid beetles, each ovary in this species consists of about twenty acrotrophic ovarioles. Mature ovarioles measure about 0·8 mm. and contain three or four developing ova. The terminal filaments arising from the anterior ends of the ovarioles join with each other to form the ligament and then the two ligaments join to form the median ligament which is attached to the abdominal diaphragm. This helps to keep the ovarioles in position.

By separate apertures all the ovarioles of each side open into a wide thin-walled egg calyx which often contains a mature ovum. When empty, its wall is considerably infolded. The egg calyx continues behind into the short and narrow lateral oviduct. The walls of the egg calyx and the lateral oviduct are composed of a single layer of gland cells, those of the former appearing to be much more contractile. Muscles and chitin have not been noted in these.

The common or median oviduct runs posteriorly below the bursa copulatrix to communicate with the vagina and, save a short anterior part, most of it lies in close association with the bursa copulatrix and the spermathecal ducts to form the massive muscular genital complex. The wall of the common oviduct is also composed of a single layer of tall highly contractile gland cells arranged on a tough basement membrane. In its posterior part, however, it is surrounded on its sides by bands of longitudinal muscles which also extend above and below it in the hinder-most part.

The bursa copulatrix is an antero-posteriorly elongated sac lying dorsally to the common oviduct and opening posteriorly into the vagina. It is lined internally by a well-developed layer of chitin produced into many irregular projections while the wall itself is made of a layer of hypoderm surrounded by a fairly thick layer of muscles. Some of muscle fibres also

surround the common oviduct ventrally and the rectum dorsally while a few bind the bursa with the spiculum gastrale. The bursa opens posteriorly into the vagina.

The spermatheca of *Laria* resembles that of other bruchid beetles and that of *Sitophylus oryzæ*. It is a strongly chitinous U-shaped capsule situated anteriorly and dorsally to the bursa with its arms directed backwards. The end of one arm is slightly broad while that of the other is tapering. The chitinous wall is feebly striped on its internal surface and muscle fibres extend across its two arms. The spermatheca is filled with spermatic fluid. It also receives the secretion of a mass of few large hyaline, unicellular glands surrounding its base and of the associated spermathecal gland.

The spermathecal gland is similar to that seen in other beetles but is comparatively smaller. It is a highly coiled, pear-shaped structure about 0·36 mm. long together with its duct. It has a large internal cavity surrounded by a single layer of cells except in the apical part where many layers can be seen. There is a prominent limiting membrane outside and a thin layer of chitin within. In the duct part, the chitinous lining is more prominent. The ducts opens in one of the arms.

The spermatheca gives off a long delicate spermathecal duct which runs ventrally and posteriorly to the bursa along the dorsal wall of which it runs for a short distance to open ultimately into the vagina at the same place at which the latter communicates with the common oviduct and the bursa. The duct is composed of a single layer of cells with chitin within. There is no trace of gland cells piercing the chitinous lining in the walls as reported by Mukerji and Bhuya (1937).

The vagina of *Laria* is a rather wide chamber not distinguishable from an 'atrium' as in some other cases. As stated before, it communicates with the common oviduct, the bursa and the spermathecal duct nearly at the same plane at its anterior end. The opening of the oviduct is situated ventrally, that of the spermathecal duct dorsally and of the bursa in between the two. It has a strongly muscular character and is lined internally by a well-developed layer of chitin. The hypodermal cells situated beneath the chitin have lost their individual entities although their nuclei are distinct. The muscles surrounding the vagina control its expansion and contraction during copulation and egg laying. Posteriorly, it is surrounded by a chitinous ring formed of the last sclerites.

The ovipositor.—As in the other two cases, the last three segments are telescoped inside so that the seventh is the last externally visible segment while the animal is at rest. The eighth tergite lying within the seventh makes

a half ring-like structure with its two anterior corners produced into a pair of notches. The posterior border of the eighth sternite is produced into a pair of strongly serrated chitinous projections. Bristles are present on both the tergite and the sternite of this segment. The ninth segment is very weakly chitinised. The tergite is posteriorly protruded into a short tongue-like process while the sternite bears a pair of strongly chitinous conical lobes, *i.e.*, the ovipositors. The ovipositors are paired, each consisting of a coxite bearing at its apex a small, considerably reduced cylindrical stylet. The coxite as well as the stylet bears a number of minute setæ. The valvifers are not distinguished from the ninth sternite on account of weak chitinisation.

The spiculum gastrale is a thin chitinous rod, situated ventrally in close association to the genital complex and extending anteriorly upto the level of the bifurcation of the common oviduct. It is bound intimately with the common oviduct and the genital complex by strong muscle fibres some of which also connect it with the ventral body wall. By the expansion and contraction of these muscles, the spiculum can be bent ventrally, causing a contraction of the lumen of the oviduct which helps regulation of the passage of ova down the oviduct. It also acts as a lever in the protrusion of the ovipositor.

Discussion

The ovaries in the Coleoptera show a considerable degree of uniformity of structure, being built, according to Berlese's classification (1910), on the acrotrophic pattern. In this respect, the present observations confirm those of Degner (1914), Imms (1924), Snodgrass (1935), etc. As regards the number of ovarioles in the ovary, while beetles differ considerably among themselves, yet, on the whole, they show specialization by reduction. Tanner (1927) in the course of a review of the genitalia of sixty-seven families of Coleoptera considered Scarabidæ as the most specialized family because it has the smallest number of ovarioles in each ovary. Among the forms studied, *Laria* has twenty ovarioles, *Rhizopertha* has eight and *Sitophylus* has two ovarioles. Thus specialization by reduction is also evident in the forms studied, specially in the latter two. In *Passalus cornutus*, Krause (1946) also reported two ovarioles and in *Aceranthus confinis*, Berlese (1910) reported only one ovariole in each ovary. Recently, Srivastava (1952) reported in *Onitis distinctus* a single ovary with a single ovariole. As a rule, the two ovaries are symmetrical in structure and position except, rarely, as in *Rhizopertha* where one of the ovaries is placed more anteriorly or as in *Onitis*, where one ovary is completely suppressed.

In regard to the histological structure of the ovarioles, one may recall the description of muscle fibres by certain workers. Thus Bordas (1900) reported a muscle layer outside the tunica propria. Imms (1938) stated that a muscle reticulum is often present in the peritoneal sheath and Krause (1946) stated that layers of circular and longitudinal muscle fibres are present outside the tunic and the epithelial layers. Krause also regarded both the layers to be smooth although muscles in the insects have been described only as striated (Morrison, 1928). In none of the cases examined has the author noted muscle fibres and it is very likely that the muscle 'reticulum' reported earlier is really a network of connective tissue and trachea.

Korschelt (1924), later supported by Mukerjee and Bhuya (1937), divided the paired efferent ducts into three parts—an anterior part, a long middle part and a short terminal part which the former considered to be of ectodermal origin. The author does not find any justification for such a division either from his studies or from the diagrams of these workers. Daviault (1928) calls the entire paired duct as 'oviduct' while Zacher (1930) designates it as the 'egg calyx'. In all the cases examined, the paired ducts are clearly differentiated into only two parts—the anterior wider egg clayx and the posterior narrow lateral oviduct. There is a distinct, though slight structural difference between the two in all cases and occasionally a clear constriction demarcates one from the other. In *Tribolium*, the lateral oviduct is lined internally by chitin. In none of the cases, chitinous hairs, as reported by Korschelt, Krautwig and Mukerji and Bhuya have been observed. In the diagrams given by the above workers, the posterior part takes a forward turn, but this in itself does not justify its distinction from the median part. Besides, there is increasing evidence that a greater part of the paired duct is ectodermal and so to consider only a short posterior part as ectodermal does not appear feasible. The presence of muscle fibres, reported by Bordas (1900) and Krause (1946) has also not been noted by the author in any of the beetles examined by him.

Daviault (1928) says that the two oviducts join to form the 'vagina' while Zacher (1930) designates the common duct formed by the union of the two egg calyces as the 'oviduct' which opens into the vagina. Among the workers on Coleoptera, Singh Pruthi (1924) labels the median duct as the uterus. While it may vary in its length or even in its histological structure from species to species, it is clear that it neither lodges the fertilised or developing ova nor does it take part in copulation. It only acts as a passage for the ripe ova down into the vagina, hence the terms 'uterus' or 'vagina' used for it are both incorrect and misleading and it should be called as median,

common or unpaired oviduct. At its posterior end, it communicates with the vagina, while the spermatheca opens at the junction of the two either directly or through the bursa.

The remaining parts of the efferent passage, *i.e.*, the spermatheca, bursa copulatrix and vagina, whose relationship with each other may vary greatly in different cases, together make a complex structure and are often jointly termed as the 'genital complex'. Of these, the spermatheca is the one which is of permanent occurrence but of very varied form. It is U-shaped in *Sitophylus* and the bruchids, cup-shaped in *Rhizopertha* and coiled, tubular in *Tribolium*. In all cases, however, it is strongly chitinous. Its relationship with other organs also varies. In *Sitophylus* and the bruchids, it opens at the junction of the oviduct and vagina by a long, slender duct. This variation in the form and position of spermatheca reminds one of Nel's remark (1929) that it is an organ of greater functional than structural significance.

The wall of the spermatheca is devoid of gland cells and the secretory activity connected with storage of sperms is relegated to one or more spermathecal glands connected with it. This spermathecal gland is also very varied in its size and structure in various beetles, being represented by a very small mass of cells in *Sitophylus* and consisting of two glands in *Rhizopertha*. The relationship of these glands with the spermatheca also varies. In *Sitophylus* and the bruchids, it opens directly in the spermatheca while in *Rhizopertha* and *Tribolium* it opens close to the latter's opening into the bursa or the spermathecal duct. In *Tribolium*, the opening has chitinous valves. The nature and function of the secretion of this gland is not properly known. Mukerji and Bhuya suggest that, being of a thin consistency, it mixes with the stored spermatozoids when the latter flow down, thinning down the spermatic fluid before fertilization. In that case, the secretion would never pass into the spermatheca as for example in *Tribolium*, while in *Sitophylus* or the bruchids it will unnecessarily increase the bulk of spermatic fluid stored in the spermatheca. To the author, it appears that the secretion does not only reduce the consistency of spermatic fluid but it primarily acts as a preservative, and possibly a nutrient of spermatozoids while they are stored in the spermatheca. In *Tribolium*, the presence of valves at the opening of the gland is also explained by this presumption. When spermatic fluid of the male passes into the bursa, its walls are distended, thereby opening the valves when the secretion also comes into the bursa and along with the sperms passes into the spermatheca.

The posterior, unpaired passage has been differentiated by some workers into two parts, *i.e.*, vagina and genital atrium. It has been shown in *Tribolium*

and *Laria* that there is no true distinction between these two regions, and in *Rhizopertha*, there is an unusually long tube, part of which is enclosed together with the rectum into an elongated chitinous tube. The posterior part is often certainly wider but this alone does not justify the distinction referred to nor do the accounts of development available to us.

The ovipositor.—From the study of the ovipositors in beetles, it is clear that in comparison to the ovipositors in other orders those of beetles are very simple and reduced structures and involve but one abdominal segment. On a study of the account of development of these in beetle [Singh Pruthi (1924), Metcalfe (1933), Rakshpal (1942)] it is clear that these represent the cavities of the ninth abdominal segment and hence are equivalent to the posterior ovipositor lobes of other orders. Therefore, the 'genital palps', 'vaginal palps' or sensory setæ (Metcalfe, Krautwig, Zacher, Mukerji and Bhuya) described on the ovipositors really represent the stylets. However, as Verhoeff pointed out long ago, the ovipositors are modified according to their function and in *Rhizopertha*, the modification is carried to an extreme with all the parts, except the palpiform styli, fused and indistinguishable from each other.

SUMMARY

The female reproductive organs of *Sitophylus oryzæ*, *Rhizopertha dominica* and *Laria affinis* have been described.

The ovaries are uniform in structure, but the number of ovarioles in the different cases varies. *S. oryzæ* has two ovarioles, *R. dominica* has eight ovarioles and *L. affinis* has about twenty ovarioles in each ovary.

The paired oviducts are distinguished into an anterior dilatable egg calyx and a posterior lateral oviduct. The walls of the two regions are histologically distinguishable, although both are composed of a single layer of cells. Muscle fibres are absent in both the regions, as also in the ovaries.

The common oviduct is a short tube leading into the vagina. In *S. oryzæ* and *L. affinis*, it is intimately associated with other organs to form the 'genital complex', while in *R. dominica*, it remains separate. It has a glandular internal lining which, in the former two, is surrounded by muscles.

The junction of the common oviduct and the vagina is marked by the opening of the spermathecal duct. This duct is thin and long lined internally by chitin in *S. oryzæ* and *L. affinis* but short and comparatively thick in *R. dominica*. In all the cases, its wall is composed of a single layer of cells.

The spermatheca is a small, hollow chitinous structure of different shapes. It is U-shaped in *S. oryzæ* and *L. affinis* and cup-shaped in *R. dominica*. In the former two, muscle fibres extending between the two arms possibly cause the expulsion of its contents. The spermathecal gland, associated with the spermatheca in *S. oryzæ* is very small and pours its secretion in the spermatheca; in *L. affinis*, it is long and opens in the spermathecal duct while in *R. dominica*, there are two glands—a small, fan-shaped gland opening into the spermatheca and a large gland opening into the duct. The secretion of the glands possibly acts as a nutriment and a preservative for the stored spermatozoids.

The bursa is a pouch-like structure in *S. oryzæ* and *L. affinis*. It is absent in *R. dominica*. In the latter, its absence is compensated by the long folded vagina. In the former two, the vagina is a strongly muscular, shallow cavity.

The spiculum is a strong chitinous bar controlling the movement of organs of copulation and oviposition.

The ovipositor is a well-developed structure consisting of paired coxites and stylets in *S. oryzæ* and *L. affinis*. In *R. dominica*, it is extremely reduced, being represented by a pair of small, palpiform stylets. In the latter, a peculiar long chitinous tube encloses the posterior part of the vagina and the rectum.

On the basis of the structure of the reproductive organs, the Curculionidæ and the Bruchidæ seem to be closely related.

ACKNOWLEDGEMENT

The author expresses his indebtedness to Dr. D. R. Bhattacharya, Ex-Professor of Zoology and Ex-Vice-Chancellor for his guidance and criticisms and to the authorities of the Indian Council of Agricultural Research for the grant of a research fellowship, during the tenure of which the work was done.

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STUDIES ON THE REPRODUCTIVE ORGANS OF FOUR SPECIES OF COLEOPTERA*

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Introduction

The morphology of the reproductive organs plays an important role in determining the taxonomy and phylogeny of insects, and a detailed knowledge of the reproductive organs and the processes is likely to throw light on the egg-laying habits and thus may be helpful in suggesting ways for checking the multiplication of insects.

The study of the reproductive system in Coleoptera seems to have attracted the attention of the entomologists only about a hundred years ago. The first outstanding work on record is of Stein (1847), who worked on the internal genitalia of some female beetles. Later Escherich (1896) and Verhoeff (1893, 1894, 1918) also added to the knowledge. Sharp and Muir (1912) and Muir (1918, 1919, 1924) worked on male beetles. Tanner (1927) made an extensive study of the Coleoptera. Blaisdell (1932, 1936) emphasized the importance of external genitalia in establishing relationship and Phylogeny. Recently Snodgrass (1936) did valuable work on the morphology of genitalia in Coccinellidæ. Mukerji and Bhuya (1937) established the affinities of Bruchidæ with Chrysomelidæ. Hinton (1940) studied the reproductive system of both the sexes in different species. Kevan (1946) studied the ædeagus of *Catopidius depressus* and Krause (1946) studied the gonads of *Passalus cornutus*. Srivastava (1950) studied the morphology of *Tribolium castaneum*.

The present paper deals with the reproductive organs of the following four beetles:—

* The work formed a *Thesis* of the junior author in lieu of one paper, for the Degree of Master of Science, 1946, of the University of Allahabad, and was conducted under the guidance of the senior author.

(i) *Onitis distinctus* Lansb., the dungroller (Scarabæidæ);

(ii) *Mylabris phalerata* Pall., a phytophagous beetle (Meloidæ);

(iii) *Sternolophus decens* Zaitzea., an aquatic beetle (Hydrophilidæ);

(iv) *Pheropsophus lineifrons* Chaud, a predaceous beetle (Carabidæ).

The dungroller was collected from the heaps of cowdung during July to November, the period during which it is available in plenty. The phytophagous beetle was collected from Malvaceous and Cucurbitaceous plants during July to December but it is available in plenty only during July and August. The aquatic beetle was collected from local ponds and tanks during the rainy season when it is abundant. The predaceous beetle was collected from underneath the stones and other shady places during October to December during which period it is available in plenty.

The body walls of male *Mylabris* and *Pheropsophus* were incised and the specimens left in 70% alcohol overnight to harden the ducts. The male aquatic beetle was given side incision and left for about twenty hours in 70% alcohol to allow the glands along with the ducts to harden. The female specimens were left in 1 : 3 mixture of chloroform and benzene for seven hours to dissolve fat and a subsequent treatment with 50% glycerine was found essential to remove extra hardening.

Borax carmine was used to stain the reproductive organs for making balsam mounts. The external genitalia were treated with 5% KOH solution before dehydrating them. Materials for microtomic sections were fixed in Bouin's fluid and the sections stained with Mans' Methyl Blue Eosin. Measurements were taken with the aid of an ocular micrometer and are given in the text in terms of millimetres. The diagrams were prepared with the aid of camera lucida as far as possible.

The authors are grateful to the Head of the Department of Zoology, University of Allahabad, for various laboratory facilities.

OBSERVATIONS

(i) *Onitis distinctus* Lansb.

A. *Male Reproductive Organs*

Testis.—There are two testes, each of which is a round, whitish body made up of six distinct follicles arranged in a compact mass, and extending from the second visible abdominal segment to the fourth. The testes are held in position by means of trachea only. Each testis measures about 3·4 mm. × 2·8 mm. The vasa efferentia arising as tiny ducts from the different follicles, meet in the centre to form the vas deferens. The follicles

are roundish, differing slightly in diameter, which is about 0·75 mm. on an average. Each follicle is lined by an epithelial layer and is full of spermatic fluid. This arrangement of testes and their follicles is similar to that described in *Tenebrio obscures* (Imms, 1948).

Vas deferens.—Each vas deferens is a slender whitish tube extending from the fourth visible abdominal segment to the fifth. After its origin, in the midst of the testicular follicles, it runs posteriorly towards the median line. With its fellow of the opposite side, it opens ventrally in the ejaculatory sac, slightly posterior to its tip. The diameter of the vas deferens is more or less uniform and there is practically no trace of vesicula seminalis. The total length of the duct is about 3·5 mm. and its diameter is about 0·35 mm.

Ejaculatory sac.—The ejaculatory sac is an elongated saccular structure, slightly curved and swollen at the tip. It runs obliquely side by side with the phallus from the fourth visible abdominal segment to the first. Before entering the phallus, the diameter of the ejaculatory sac is almos halved and this narrowed part may be said to represent the duct which opens into the phallus by a triangular gonopore. The length of the sac is about 2·3 mm. and its diameter about th middle is about 0·4 mm. (Fig. 1).

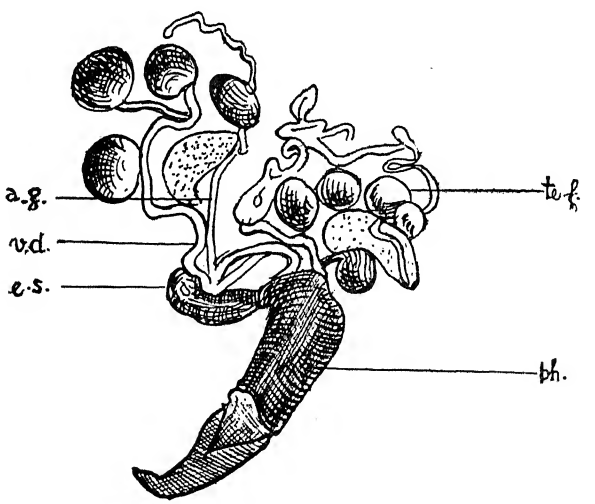


FIG. 1. Entire mount of the male reproductive organs of *Onitis distinctus.*

Accessory gland.—There is only one pair of accessory glands arranged symmetrically. Each gland is divisible into a saccular and a tubular part. The former lies transversally, mainly in the second visible abdominal segment, partly overlapping the testes and measures about 1·28 mm. × 0·48 mm,

The latter is highly convoluted, measures about 5·1 mm.×0·15 mm. and extends from the third visible abdominal segment to the fifth. The two glands of the two sides unite posteriorly in the median line and open ventrally in the ejaculatory sac just posterior to the opening of the two vasa deferentia.

The *phallus* is a hollow conical structure containing the terminus of the ejaculatory duct. In the retracted condition, it extends from the first visible abdominal segment to the sixth and lies more or less towards the right side. Its average size is about 4·8 mm.×0·8 mm., it is distinguished into a proximal part, the phallobase and a distal part, the ædeagus (*median lobe* of Sharp and Muir and *trabes* of Verhoeff .

Phallobase.—The phallobase is made up of two lateral basal plates which meet in the middle (Fig. 2). Laterally from the phallobase arise two

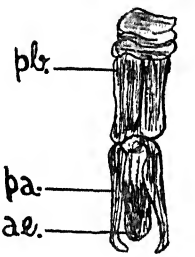


FIG. 2. Entire mount of the phallus (KOH preparation) of *Onitis distinctus.*

parameres as elongated triangular plates, thicker towards the proximal end and tapering towards the distal end. Their terminal portions are turned slightly inwards.

Aedeagus.—The ædeagus is a tubular, elongated structure between the two parameres. Its broad base is supported on the phallobase. The gonopore lies at the junction of the penultimate segment and the ultimate one.

B. *Female Reproductive Organs*

Ovary.—There is a single unpaired ovary with a solitary ovariole lying ventral to the alimentary canal. This is unusual in Coleoptera. The ovary is convoluted like the letter " S " and lies symmetrically, a little towards the left, extending from the first visible abdominal segment to the fourth. The anterior part is curved and is continuous with the suspensory ligament which is attached to the dorsal wall of the diaphragm. The ovariole is acrotrophic and contains from twenty to thirty eggs. The average size of the ovariole is 14·8 mm. and it is broader towards the posterior end (Fig. 3).

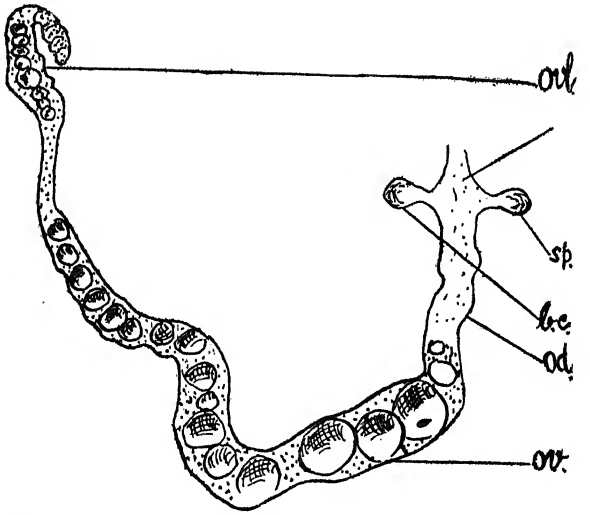


FIG. 3. Entire mount of the female reproductive organs of *Onitis distinctus*.

Oviduct.—The oviduct is a short stout tube, lying in the fifth and sixth visible abdominal segments. After the opening of the spermathecal duct into it, the oviduct widens into the vagina which terminates in the ovipositor in the sixth visible abdominal segment. It is about 0·6 mm. in length and has an average diameter of 0·6 mm.

Spermatheca.—The spermatheca is an oval dirty brown body about 0·8 × 0·3 mm. in size, lying in the fifth visible abdominal segment. The spermathecal duct opens into the vagina in the median line.

Bursa copulatrix.—The bursa is sac-like oval deep brown body and in natural condition lies slightly to the left in the fifth visible abdominal segment.

The presence of a single ovary with one ovariole in this beetle brings up a number of interesting points. The most important of these are (i) does this condition occur in any other species of the genus and other families of Coleoptera, (ii) how has this condition been brought about in this beetle, and (iii) whether the occurrence of a single ovary with one ovariole is a primitive or a secondary feature?

The occurrence of paired ovaries is a common feature among insects. Amongst the Apterygota, in Thysanura, there are two ovaries each having five to seven ovarioles. Among the Pterygota, two ovaries occur in the lower orders such as Orthoptera as well as in some higher ones—Hemiptera, Coleoptera, Hymenoptera and Diptera. As regards the primitive number of ovarioles, most probably it is never more than eight—this number is retained in *Periplaneta* among the Orthoptera. In certain Diptera such

as *Melophagus* and *Hippobosca* there are two ovaries with two ovarioles to each. Certain viviparous Diptera, producing a small number of relatively large eggs, have two ovaries, each with a single ovariole. The occurrence of a single ovary with one ovariole is a common feature in certain Aphididæ (Hemiptera) where it is a case of specialisation by reduction.

Generally speaking, in insects, specialisation by multiplication is more frequent. Thus among the Diptera, in *Calliphora* and in *Hypoderma* there are one hundred or more ovarioles to each ovary. The maximum number of ovarioles in the species of *Termes* (Isoptera) is reported to be more than twenty-four hundred. Tanner (1927), as a result of his study of sixty-seven coleopteran families, has concluded that Scarabæidæ is the most specialised family. From this it is inferred that the presence of a single ovariole in the beetle under consideration, is a specialisation brought about by reduction in the number of ovarioles, and as regards the other point further investigation is necessary.

Ovipositor.—The ovipositor is a short chitinised tubular structure occupying the sixth visible abdominal segment formed by fusion of the pieces of which it is made—(the shaft and the basal apparatus) (Fig. 4). It is bent

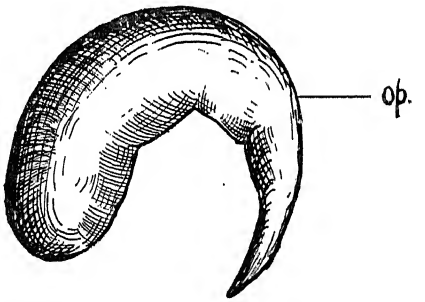


FIG. 4. Entire mount of the ovipositior (KOH preparation) of *Onitis distinctus*.

upon itself near the middle and its tip is gradually drawn into a pointed portion slightly curved inwards. It measures about 0·6 mm. × 0·13 mm. on an average and opens at the commencement of the seventh segment.

(ii) *Mylabris phalerata* Pall.

A. *Male Reproductive Organs*

Testis.—The two testes lie symmetrically on either side of the alimentary canal in the fourth and the fifth visible abdominal segments. Each testis forms a compact mass and is more or less ovoidal in shape, being more convex dorsally. It is attached to the intestine by means of tracheal branches

and is about 2·5 mm. × 1·75 mm. in size. From a notch in the middle on the ventral side, the vas deferens arises.

A notable feature about the testes of this species is that in one series of microtomic sections, one of the follicles approached in appearance an

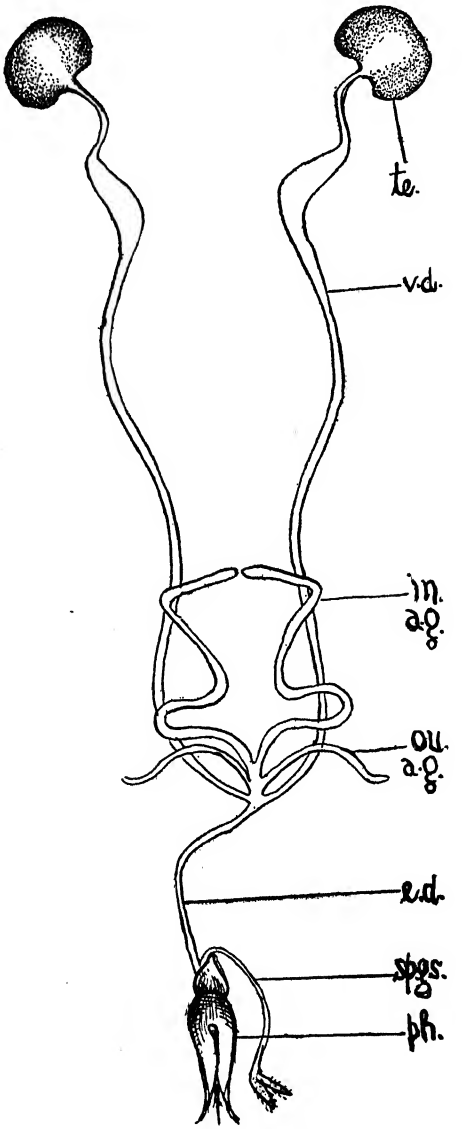
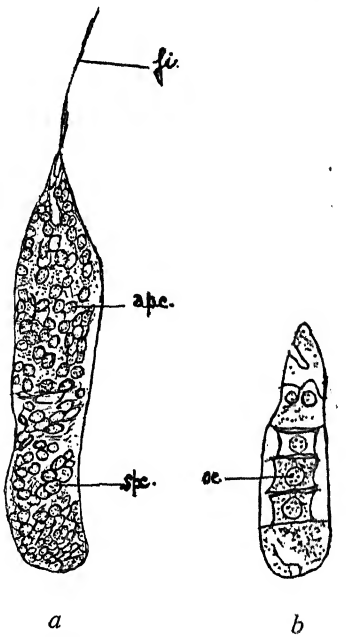


FIG. 5. Entire mount of the male reproductive organs of *Mylabris phalerata.*

ovarian rather than a testicular follicle (Fig. 6, *b*.) Several large ova-like structures were visible, arranged in a single line, each with a prominent

nucleus in the middle. In some cases, giant spermatid formation is known to occur but the probability of this happening in this case is ruled out by the typical ovarian arrangement seen in the few large cells developing into ova-like structures. Probably this follicle was to produce ova and it may be a case of partial gynandromorphism. Further investigation is likely to reveal the occurrence of gynandromorphs in this species.



a *b*

FIG. 6. *a* and *b*. L.S. of the testicular follicles of *Mylabris phalerata*.

Vas deferens.—Each vas deferens is a slender tube with its diameter varying from place to place and having a rather complicated course. At its origin in the sixth visible abdominal segment it is very slender, but after running for about 12 mm. it takes a turn towards the median line and runs posteriorly making a thicker loop after which its diameter again decreases and remains almost uniform for the rest of its course. After the formation of the loop, it runs anteriorly parallel to that of the opposite side, in a straight line till it reaches the second visible abdominal segment where it gets under the dorsal loop of the accessory gland and runs postero-medially to join its fellow and form the common duct (Fig. 5). The total length of the vas deferens is about 28 mm. and its maximum diameter only about 1 mm.

Ejaculatory duct.—The ejaculatory duct is formed by the union of the two vasa deferentia. It is about 11 mm. long and approximately 0·5 mm. in diameter. It runs from the posterior border of the second visible abdominal segment to the sixth, where it enters the ædeagus.

During about half its course, the ejaculatory duct runs ventral to the vasa deferentia and takes a more or less straight course slightly to the right of the median line. Then it turns abruptly to the left as shown in Fig. 5, and joins the ædeagus. Its terminal portion near the ædeagus is slightly dilated.

Accessory gland.—Two pairs of accessory glands lie in the second and third visible abdominal segments. The outer is smaller and simpler in course being about 5 mm. long and 0·5 mm. in diameter and join with them before opening into the ejaculatory duct.

The inner are bigger and somewhat thicker than the outer ones, being about 11·12 mm. in length with an average diameter of 0·75 mm. Their maximum diameter is 1 mm. in the middle of their course. Both the inner glands are thrown into loops as indicated in Fig. 5.

Escherich (1894) classified the accessory glands into mesadenia and ectadenia according to his conception of their origin. Bordas (1900) named them as external and internal accessory glands. Bordas (1900) and Murray and Tiegs (1935) considered both the sets of accessory glands to be mesodermal. Later workers like Pruthi (1924, 1925), Metcalfe (1932) and Srivastava (1950) regard them as ectodermal in origin.

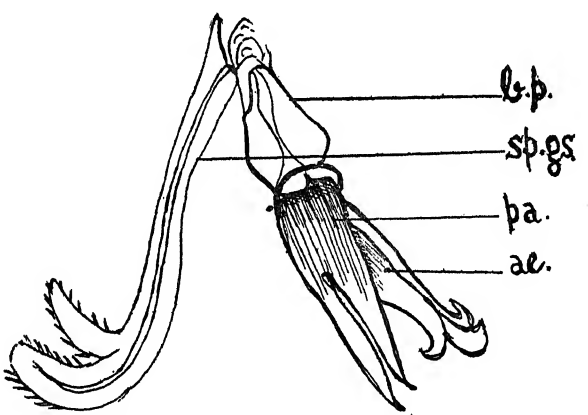


FIG. 7. Entire mount of the phallus (KOH preparation) of *Mylabris phalerata.*

The *phallus*, as usual, is a chitinised hollow structure. In the retracted condition it lies in the sixth and seventh visible abdominal segments measuring about 7 mm. × 0·38 mm. It is made up of a phallobase and an ædeagus (Fig. 7).

Phallobase.—The phallobase is made up of two triangular pieces, the basal plates, which are flattened disc-like structures.

Aedeagus.—The ædeagus arises from the base of the basal plates. It is a stout hollow rod bearing three sharp hooks at its extremity. The presence of the hooks in the ædeagus of this species is remarkable. Ventrally to the ædeagus there is a thin long chitinous rod the spiculum gastralæ which works as a support to the various muscles controlling the movement of the phallus. The spiculum is slightly bent anteriorly and bifid posteriorly. The bifid part serves for the attachment of the various muscles already referred to before.

B. *Female Reproductive Organs*

Ovary.—The paired ovaries each measuring about 8 mm.×4·5 mm. and extending from the second to the fifth visible abdominal segments, lie symmetrically ventral to the alimentary canal.

Each ovary looks like a bunch of elongated grapes consisting of numerous acrotrophic ovarioles each of which is more or less elongated with three clearly developing eggs inside it. It is usually about 2 mm.×1·5 mm. at the level of the oldest egg. The proximal ends of ovarioles, on each side, are produced into thread-like filaments (Fig. 8) which unite to form right and left suspensory ligaments attached to the posterior wall of the metathorax.

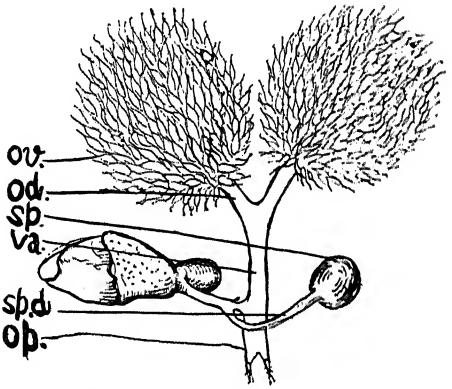


FIG. 8. Entire mount of the female reproductive organs of *Mylabris phalerata.*

Oviduct.—The lateral oviducts are short, fairly thick, measuring about 2·75 mm.×0·75 mm. on an average, and converge to form the common duct. The common oviduct measures 2 mm.×0·75 mm. and lies in the fifth visible abdominal segment.

Vagina.—The vagina is the slightly swollen posterior part of the oviduct, not very distinct from it, and is about 1 mm. wide and into which opens the bursa as shown in Fig. 8.

Spermatheca.—The spermatheca is an unpaired ovoid structure measuring 1·2 mm. × 1·0 mm. on an average and lying in the fifth visible abdominal segment. From its ventral side arises the spermathecal duct which after running for a short distance opens into the bursa before the latter joins the vagina.

Bursa copulatrix.—The bursa is a sac-like ovoidal structure with a knob-like protrusion towards the inner end. It lies slightly to the left of the vagina and dorsal to the alimentary canal, and extends from the middle of the sixth visible abdominal segment to the fourth. It opens into the vagina by a short narrow duct and measures nearly 5 mm. × 2 mm.

Ovipositor.—The ovipositor is a paired chitinised structure in continuation with the vagina and occupies a part of the sixth and seventh visible abdominal segments. Each is made up of the shaft and the basal apparatus.

Shaft.—The shaft in most insects is made up of two pairs of closely oppressed elongated processes, the first and the second pair of valvulæ. In this case, the valvulæ of each pair are completely fused to form a single process which is supported on the basal apparatus by a rounded end. It is beset with a number of tiny bristles and spines (Fig. 9). In size it is 0·46 mm. × 0·2 mm.

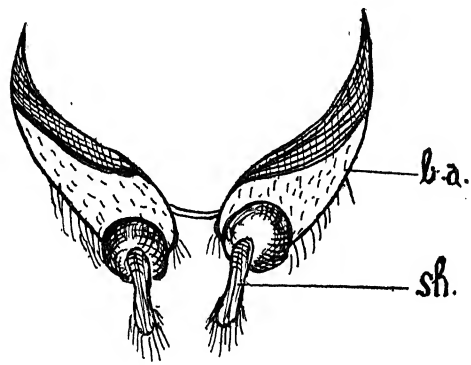


FIG. 9. Entire mount of the ovipositor (KOH preparation) of *Mylabris phalerata.*

Basal apparatus.—The basal apparatus consists of two curved and pointed plates, each of which represents the fused first and second valvifers of its side, the line of fusion of which is distinguishable.

It may be added that the shaft and the basal apparatus in this case are quite distinct, unlike those of *Onitis* where they have completely fused. The genital opening lies at the tip of the last visible abdominal segment.

(iii) *Sternolophus decens* Zaitzea

A. *Male Reproductive Organs*

Testis.—The two testes lie symmetrically on either side of the alimentary canal, each being a round compact body enclosed in a thin vesicle and held in position by means of fine trachea. It is about 0·7 mm. in diameter and consists of a bunch of many small follicles occupying the posterior portion of the second visible abdominal segment. Each follicle is an elongated structure measuring about 0·68 mm. × 0·08 mm. From the meeting point of all the follicles in the centre, arises the vas deferens.

Vas deferens.—Each vas deferens is a thin slender tube about 2·38 mm. long and with almost a uniform diameter of 0·07 mm. It lies in the second and third visible abdominal segments. After its origin from the testis it runs postero-medially and crossing the accessory glands it meets its fellow to swell up near the posterior border of the third visible abdominal segment and form the vesicula seminalis. The latter is a saccular structure being about 1·27 mm.×0·17 mm. in size. The distal ends of the two vesiculæ seminalis unite to form the ejaculatory duct.

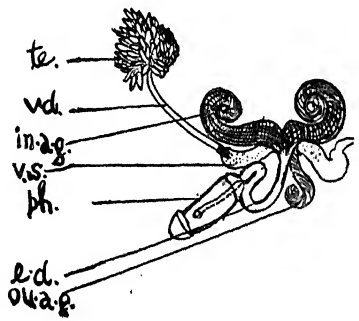


FIG. 10. Entire mount of the male reproductive organs of *Sternolophus decens*.

Ejaculatory duct.—The ejaculatory duct is a slender tube running posteriorly through the third and fourth visible abdominal segments. It is about 2·8 mm. long and 0·17 mm. wide. A short distance from its origin it is somewhat swollen; but it becomes thinner again and after making two loops terminates in the ædeagus. The ejaculatory sac seems to be absent in this case.

Accessory gland.—There are two pairs of accessory glands, the outer pair and the inner pair. They extend from the third visible abdominal segment to the first.

The outer pair is the larger and the thicker one. Posteriorly its diameter goes on increasing gradually until the maximum is reached near its opening into the anterior expanded part of the ejaculatory duct. The anterior free end is thrown into a double coil towards its inner side like a watchspring (Fig. 10). Regular transverse wrinkles are clearly visible on its entire length giving it a ringed appearance. This is due to well-developed circular muscle fibres in its wall. The length of each gland is about 2·4 mm. and the maximum diameter about 0·37 mm.

The inner pair resembles the outer one in appearance and like them their anterior parts are also thrown into double coils. They are also thickest at the points where they open into the anterior expanded part of the ejaculatory duct—the opening being slightly anterior to that of the outer glands. The length of each tube is about 2·13 mm.

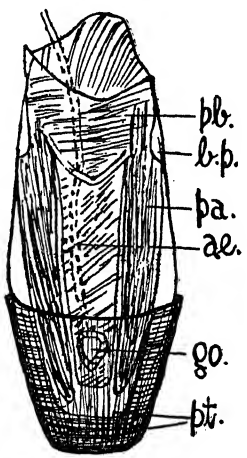


FIG. 11. Entire mount of the phallus (KOH preparation) of *Sternolophus decens*.

The *phallus* is a chitinised tubular structure. In the retracted condition, it is overlapped by the intestine and occupies a central position in the fifth and sixth visible abdominal segments. It is about 1·87 mm. long and about 0·49 mm. wide and is distinguishable into a phallobase, an ædeagus and a phallotheca.

Phallobase.—The phallobase is made up of two basal plates which occupy a lateral position and are very much reduced, being almost of the shape of an equilateral triangle. From the phallobase arise laterally the two parameres as straight rods of more or less uniform diameter. Thus they differ in form and size from those of the beetles described in previous chapters.

Aedeagus.—The ædeagus is a median tubular structure between the two parameres. It is broadest at the base and gradually tapers towards the extremity. Its base carries two short pointed spines directed anteriorly and its extremity extends beyond the gonopore. The gonopore is triangular and bears numerous transverse ridges on its margin. It opens to the outside at the end of the last segment.

Phallotheca.—The phallotheca arises as a fold from the phallobase along its ventral margin and extending beyond the ædeagus and the parameres turns forwards dorsally to form a surrounding cup-like envelope or sheath around the distal third of the ædeagus and the parameres (Fig. 11).

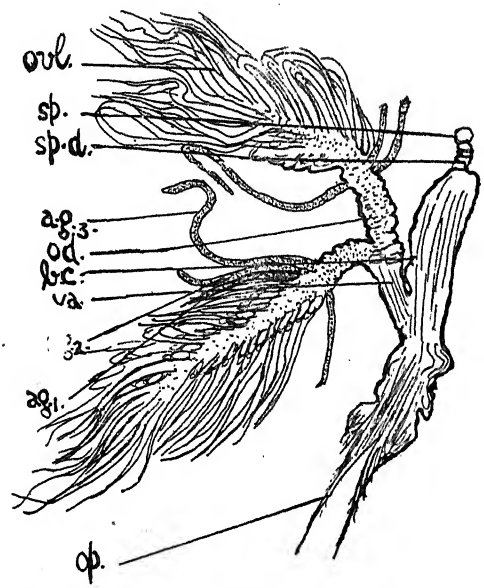


FIG. 12. Entire mount of the female reproductive organs of *Sternolophus decens.*

B. *Female Reproductive Organ*

Ovary.—The two ovaries, lying symmetrically, extend from the first visible abdominal segment to the third. Each ovary is an elongated, branched structure made of many long acrotrophic ovarioles (Fig. 13) and measures about 2·72 mm.×0·56 mm. Two large eggs occur in each ovariole towards its distal end and its proximal end is an elongated chamber with numerous nurse cells mixed with some undifferentiated egg cells. Each ovariole measures about 0·68 mm.×0·03 mm. An interesting feature is the method in which the ligaments from different ovarioles combine to form the suspensory ligaments. The ligaments of some ovarioles unite together in groups and the several ligaments thus formed ultimately join to form the

common suspensory ligament which attaches itself to the diaphragm of the metathorax. From the base of each ovary arises the lateral oviduct.

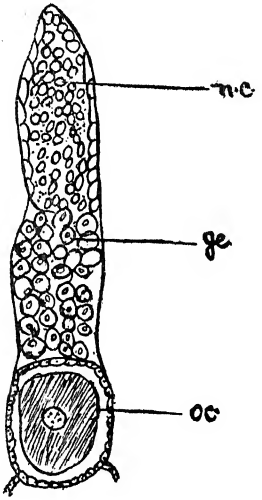


FIG. 13. L.S. of the ovariole of *Sternolophus decens*.

Oviduct.—The two lateral oviducts lie in the fourth visible abdominal segment and run almost transversely towards the median line to meet and form the common duct. Each lateral oviduct measures about 0·39 mm. × 0·25 mm.

The common oviduct is a short median tube occupying a portion of the fifth visible abdominal segment and measures about 0·68 mm. × 0·15 mm. Later on it swells to form the vagina.

Vagina.—The vagina is a short tubular structure occupying a part of the fifth visible abdominal segment. It is about 0·68 mm. long and 0·29 mm. in diameter.

Spermatheca.—There is a single spermatheca the right one as in *Mylabris*. It is a small dome-shaped piece lying in the posterior third of the fourth visible abdominal segment. The spermathecal duct arises from its base and after a short sinuous course opens into the bursa at the tip.

Bursa copulatrix.—The bursa is an elongated saccular structure running anteriorly from the middle of the fifth visible abdominal segment to the third. It has the maximum breadth (0·85 mm.) in the middle, the usual length being about 2·12 mm. It opens into the vagina about the middle of its length by a short narrow duct.

Accessory gland.—There are three pairs of sub-equal accessory glands, which are tubular and located in the fourth and the third visible abdominal segments. Each is about the length of the ovary with a diameter of about 0·17 mm. They open in the lateral oviduct at different planes (Fig. 12).

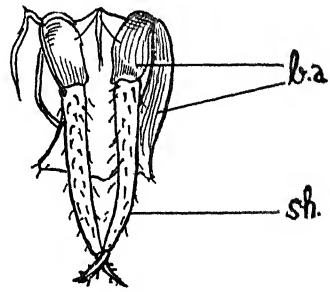


FIG. 14. Entire mount of the ovipositor (KOH preparation) of *Sternolophus decens.*

Ovipositor.—The chitinised ovipositor when retracted lies in the fifth visible abdominal segment, and consists of a shaft and a basal apparatus.

Shaft.—The shaft consists of two elongated processes formed by the fusion of the members of the first and the second pair of valvulæ. Each process shows clear transverse chitinisation which seems to divide it into three parts. A short broader basal, a narrower much elongated median and a short curved distal piece. It is beset with bristles practically all over.

Basal apparatus.—The basal apparatus consists of two pieces which are flattened leaf-like triangular structures with the apex pointed. Each piece is made up of a pair of valvifers, the fusion between the members of each pair having taken place only two pieces are visible (Fig. 14).

(iv) *Pheropsophus lineifrons* Chaud.

A. *Male Reproductive Organs*

Testis.—There are a pair of testes forming a compact rounded mass of about 2 mm. in diameter (as in *Mylabris*) lying symmetrically on either side of the alimentary canal in the fourth and fifth visible abdominal segments. Each testis is coloured bright yellow and held in position by means of tracheal tubes. The vas deferens arises ventrally about the middle part of each testis.

Vas deferens.—Each vas deferens is very slender at its origin but gets slightly thicker in its posterior part. It originates in the fourth visible abdominal segment and after running forwards for about 4 mm. forms a

loop and receives the accessory gland. Apparently it appears as if the vas deferens opens into the accessory gland.

Ejaculatory duct.—The two vasa deferentia unite in the median line to form the narrow winding ejaculatory duct which continues into the base of the ædeagus. It measures about 1·62 mm.×0·32 mm.

Accessory gland.—There is a single pair of long tubular accessory glands, the outer, as is the case in other ædephagous forms. In its natural condition each gland is bent one upon itself transversally and extends from the first visible abdominal segment upto the middle of the third visible abdominal segment where it joins the vas deferens (Fig. 15). The length of each gland is about 28·85 mm. and the maximum diameter about 0·44 mm.

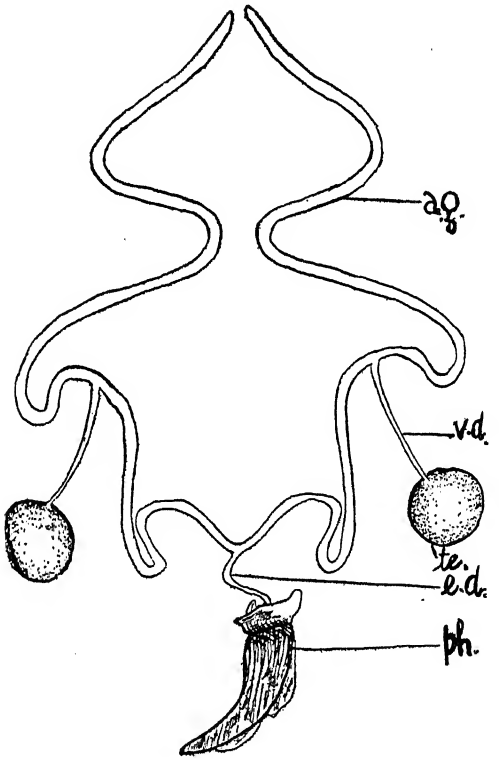


FIG. 15. Entire mount of the male reproductive organs of *Pheropsophus lineifrons.*

The *phallus* is an elongated curved chitinous structure, lying in the last two segments. It measures about 4·08 mm.×0·7 mm. and consists of the phallobase and ædeagus (Fig. 16).

Phallobase.—It is made up of two basal plates which are fairly reduced in size. Laterally from the phallobase arise two feebly chitinised parameres which are much flattened unlike those of the other three beetles.

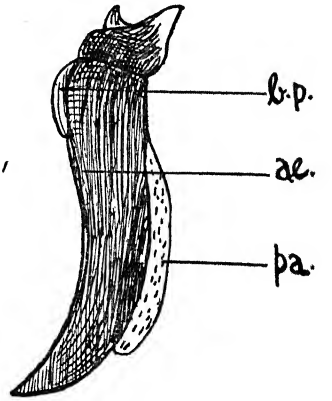


FIG. 16. Entire mount of the phallus (KOH preparation) of *Pheropsophus lineifrons.*

Aedeagus.—It is a curved highly chitinised structure occupying the seventh visible abdominal segment, and into it opens the ejaculatory duct medially. The distal end of the ædeagus is somewhat broadly pointed. The ejaculatory duct opens into the ædeagus at the end of the last visible abdominal segment by a triangular gonopore.

B. *Female Reproductive Organs*

Ovary.—A pair of ovaries lie symmetrically one on either side of the alimentary canal. Each is attached separately by a suspensory ligament to the dorsal wall of metathorax and extends posteriorly upto the fourth visible abdominal segment. Each mature ovary is about 4·25 mm. × 2·8 mm. in size.

Each ovary is fusiform in shape being broadest in the middle and consists of about fifteen short polytrophic ovarioles (Fig. 18), each with a single mature egg and measuring about 3·5 mm. × 2 mm. on an average. From the proximal end of each ovariole arises a filament, all the filaments of one side unite to form the common suspensory ligament which is attached to the dorsal body wall. At the base of each ovary all the ovarioles join together to form the lateral oviduct (Fig. 17).

Oviduct.—Each lateral oviduct is a short tube about 1 mm. × 0·35 mm. in size. It starts from the fourth visible abdominal segment and proceeds backwards towards the median line to unite with that of the other side in the beginning of the fifth visible abdominal segment and form the common

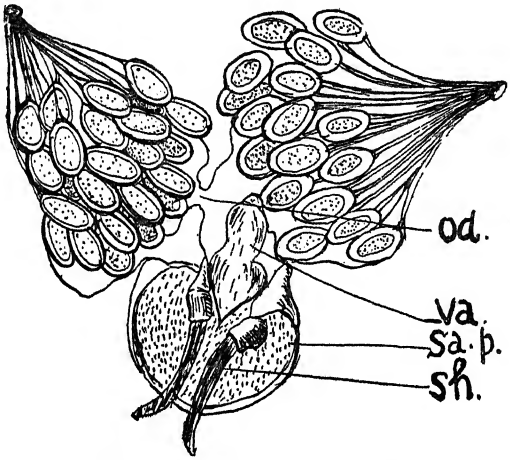


FIG. 17. Entire mount of the female reproductive organs of *Pheropsophus lineifrons.*

oviduct, which is about twice as thick as the lateral oviduct. The common duct proceeds backwards in the sixth visible abdominal segment, makes a loop and swells up to form the vagina. It measures about 2·85 mm.× 0·25 mm.

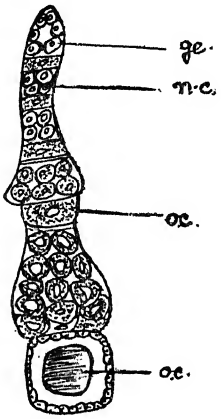


FIG. 18. L.S. ovariole of *Pheropsophus lineifrons.*

Vagina.—The vagina is a short somewhat curved structure occupying the sixth visible abdominal segment. It terminates into the ovipositor in the beginning of the seventh visible abdominal segment.

Ovipositor.—The ovipositor is an elongated chitinised structure occupying the seventh visible abdominal segment. As usual it consists of a shaft and a basal apparatus each of which is double in number.

Shaft.—Each shaft is a sword-like elongated structure fitted on a broader base produced into an anteriorly directed prominent and curved spine with serrated margin. It is made up by the fusion of the two valvulæ of its side. It is beset with bristles practically all over.

Basal apparatus.—The basal apparatus consists of two flattened, more or less triangular pieces. Each piece has an inwardly directed process which serves for the attachment of the shaft, and distally bears a small triangular piece separated from the main part by a clear line (Fig. 19). The size of the basal piece is about 2·94 mm. × 1·2 mm.

Suranal plate.—The suranal plate is a broadly flattened oval structure lying ventrally under the anterior half of the ovipositor. It is feebly chitinised except in the middle (Fig. 19).

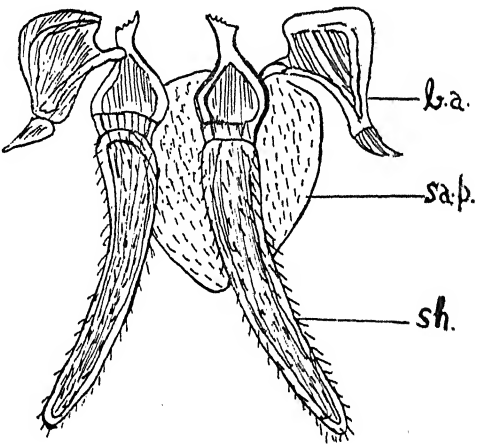


FIG. 19. Entire mount of the ovipositor (KOH preparation) of *Pheropsophus lineifrons.*

DISCUSSION

This study of the genital organs of the four species of beetles belonging to four different families, clearly indicates that the genital organs are adapted to their body forms and egg-laying habits.

In the *Onitis* species, the ovipositor is a single mass of thickly chitinised fused pieces and is sharply pointed at the tip. The reason for the fusion of the various parts of the ovipositor and for its being sharply pointed is that it lays eggs into the dung mass which it rolls into its holes. Of the other three species, *Mylabris* lays eggs on the leaves and shoots, *Sternolophus* on the surface of the leaves of aquatic plants, *Pheropsophus* on the surface of the earth where it remains hidden, hence no special device for laying eggs is needed in their ovipositors.

As for the phylogeny of these beetles, the structure of the reproductive organs in *Onitis*, particularly in the male, seems to throw some light on its phylogeny. Each testis is made up of six roundish follicles from each of which arises a vas efferens which ultimately unite to form the vas deferens. A similar arrangement of follicles is described in *Tenebrio obscurus* by Imms (after Bordas, 1900). These resemblances may be due to a closer phylogenetic affinity between the families Scarabæidæ and Tenebrionidæ.

In *Mylabris* species, the testis is a round compact mass and the follicles are arranged so closely that it is not possible to separate them. The structure of the testis of this beetle resembles that of *Pheropsophus*. The sections also of the testes of *Mylabris* and *Pheropsophus* reveal close resemblance. The Carabidæ is primitive in several respects and it is probable that *Pheropsophus* may be a connecting link between the Carabidæ and the Meloidæ.

Onitis and *Mylabris* show specialisations. In the former this specialisation seems to have taken place by reduction, as is proved by the singleness of the ovary and its ovariole, and by the fusion of the shaft and the basal apparatus of its ovipositor. But in *Mylabris* the specialisation seems to have taken place by multiplication as is evident by the presence of a large number of ovarioles.

In *Sternolophus* too there is an increase in the number of ovarioles and the phallobase has extended forwards to form a phallotheca.

Pheropsophus shows no specialisation in the reproductive organs, hence on the basis of the genital organs alone Scarabæidæ and Meloidæ seem to stand higher in the evolutionary scale, but which of the two occupies a higher position cannot be said for the present. The conclusion of Tanner (1927) that the Scarabæidæ are the most highly specialised among the sixty-seven coleopteran families studied by him may be well founded. Next in the rank is the aquatic beetle (Hydrophilidæ) and last of all stands *Pheropsophus* (Carabidæ).

Summary and Conclusion

The reproductive organs of four different families of Coleoptera, obtainable at Allahabad, are described.

In *Onitis distinctus* Lansb. the genital organs do not show any remarkable feature in the male but the female has only one ovary with a single ovariole —a feature hitherto unrecorded in Coleoptera.

In *Mylabris phalerata* Pall. the male genital organs are remarkable on account of the presence of three sharp hooks at the extremity of the ædeagus. In the female the number of ovarioles is very large.

In *Sternolophus decens* Zatzea, male, the phallobase surrounds the entire phallus. The female has three pairs of accessory glands.

In *Pheropsophus lineifrons* Chaud., male, the two accessory glands are very long. The female does not show any remarkable feature.

The structure of the genital organs in beetles seems to throw some light on their phylogenetic relationship. The structural modifications of the genital organs are in accordance with their body forms and egg-laying habits.

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ABBREVIATIONS

ae.	.. ædeagus.	*op.*	.. ovipositor.
a.g.	.. accessory gland.	*ov.*	.. ovary.
a.g. 1	.. accessory gland 1st pair.	*ovl.*	.. ovariole.
a.g. 2	.. accessory gland 2nd pair.	*ou.a.g.*	.. outer accessory gland.
a.g. 3	.. accessory gland 3rd pair.	*pa.*	.. paramere.
ap.c.	.. apical cell.	*ph.*	.. phallus.
b.a.	.. basal apparatus.	*pb.*	.. phallobase.
b.c.	.. Bursa copulatrix.	*pt.*	.. phallotheca.
b.p.	.. basal plate.	*sa.p.*	.. suranal plate.
e.d.	.. ejaculatory duct.	*sh.*	.. shaft.
e.s.	.. ejaculatory sac.	*sp.*	.. spermatheca.
fi.	.. filament.	*spc.*	.. Spermatocyte.
ge.	.. germarium.	*sp.d.*	.. spermathecal duct.
go.	.. gonopore.	*sp.gs.*	.. spiculum gastralæ.
in. a. g.	.. inner accessory gland.	*te.*	.. testis.
l. od.	.. lateral oviduct.	*te.f.*	.. testicular follicle.
m. od.	.. median oviduct.	*va.*	.. vagina.
n.c.	.. nurse cell.	*v.d.*	.. vas deferens.
o. oc.	.. oocyte.	*v.e.*	.. vas efferens.
od.	.. oviduct.	*v.s.*	.. vesicula seminalis.

A NEW HETEROPHYID TREMATODE *HAPLORCHIS RAYII*, N.SP. (FAMILY—HETEROPHYIDÆ ODHNER, 1914) FROM KITE *MILVUS MILVUS MIGRANS* *

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[Communicated by Prof. H. R. Mehra, M.SC., PH.D. (cantb.), F.N.I.]

LOOSS (1899) proposed the genus *Haplorchis* for *Monostomum pumilio* (Looss, 1896) parasitic in the intestine of *Pelcanus onocrotalus* and *Disthirinus* (Looss, 1896) parasitic in the guts of fishes, *Bagarus bayad* and *B. docmæ*. Witenburg (1930) emphasized the synonymy of the genus *Haplorchis* and *Monorchotrema* Nishigori (1924). He pointed out the unmistakable identity between their type species *H. pumilio* Looss, 1896 and *M. taihokui* Nishigori (1924). Gohar 1934 placed *Monorchotrema taichui* Nishigori (1924) as *Haplorchis taichui* Nishigori, (1924). This view was supported by the works of Mueller and Van Cleave (1932), Tubangui (1933), Manter (1934) and Chen (1936). Srivastava (1935) transferred *H. anguillarum* Tubangui (1933) to a new genus *Tubanguia* owing to the presence of two testes in it. He also splitted the genus *Haplorchis* into two sub-genera: *Haplorchis* (Monorchotrema) and *Haplorchis* (Haplorchis). He assigned *H. taichui*, *H. microrchis* and *H. yokogawai* with *H. pumilio* as its type-species to the sub-genus *Haplorchis* (Monorchotrema) and to the latter sub-genus he placed *H. attenuatum* Srivastava (1935), *H. piscicola* Srivastava (1935), *H. gangeticum* Srivastava (1935), *H. silundii*, Srivastava (1935) with *H. cahirinus* Looss, 1935 as the type-species. Dayal (1935) found *H. taakree* in an Indian Freshwater fish. Chen (1936) further accepted and asserted that the subfamily Haplorchinæ and the genus *Haplorchis* are valid while the subfamily Monorchotreminæ and the genus *Monorchotrema* are synonyms. Chen (1949) also created the genus *Haplorchoides* out of the six species formerly included in the genus *Haplorchis*. He stated that the *Haplorchoides* has longer pre-pharynx, short œsophagus and a more anterior location of vitellaria and testis than *Haplorchis*.

Ten specimens were found in an examination of the intestine of Common Pariah-Kite, *Milvus milvus migrans* shot at Mukteswar (Kumaon). The trematodes are oval, very small in size measuring 0·797 mm. in length and 0·674 mm. in breadth. The fluke has a rather thick hyaline homogeneous

and transparent integument. The surface of the body is thickly covered by projecting spines which are recurved and gradually reduced both in size and number as they proceed from oral to distal part of the body till there are no spines in the hinder one-fourth part.

The oral sucker is sub-terminal, muscular, situated at the anterior end of the body. It is oval, broader than long, measures 0·049 mm. in length and 0·066 mm. in breadth. The acetabulum is absent. There is no prepharynx and the oral sucker immediately opens into a muscular well-developed and nearly round pharynx measuring 0·039×0·033 mm. The œsophagus is 0·125 mm. long and bifurcates to form the two intestinal cæca in front of the gonotyle. The two cæca run near the body wall and terminate behind the testis reaching the hinder end of the body.

The reproductive organs occupy the most prominent position and the most space in the posterior part of the body. The presence of a single large transversely oval testis 0·232 mm. in length and 0·305 mm. in breadth is the characteristic feature of this fluke. It occupies the posterior-most quarter of the body. The vesicula seminalis lies on right anterior side in between the testis and the ventro-genital-sinus. It is filled with sperms and measures 0·57×0·165 mm. The vesicula seminalis is clearly constricted into two oval chambers nearly of the same size. The latter chamber of the vesicula seminalis opens into small prostatic duct which leads into the ejaculatory duct. The ejaculatory duct after forming a common hermophroditic duct with the metraterm opens into the ventro-genital-sinus at its posterior right side. There is neither cirrus nor cirrus sac.

The ovary is situated on the right side of the median plane of the body in between the testis and ventro-genital-sinus. It is rounded or oval measuring 0·115×0·108 mm. The recepticulum seminis is situated on the right side of the ovary somewhat obliquely behind the latter. The recepticulum seminis is globular and measures 0·043 mm. in diameter—its size depending on the amount of its contents. Laurer's canal is given off from the junction of the recepticulum seminis and the oviduct. The vitelline glands are well developed. They are situated in the posterior part of the body beginning from the level of the ovary. They are dorsally and laterally situated forming scattered irregular groups of small follicles even covering parts of ovary and testis to some extent. The follicles join up below the testis in small elongated groups from both the sides. Many ducts unite these groups of vitellaria which in their turn combine to form the two vitelline ducts from the two sides and proceed towards the posterior margin of the ovary to form common vitelline ducts.

The uterus occupies all the available space between the other organs in the posterior part of the body. The uterus first runs posteriad and then anteriad many times in closely folded longitudinal coils until it opens into the genital sinus. The eggs in the uterus around the vesicula seminalis and below it are all mature.

The ventro-genital-sinus which is a oval depression on the ventral body surface lies just behind the intestinal bifurcation. It is rounded and measures $0 \cdot 089 \times 0 \cdot 102$ mm. It encloses the gonotyle bearing fifteen conspicuous keel-shaped spines or rodlets forming a semi-circular crown. The rodlets are chitinous in nature. The rodlets on both margins of the semi-circular crown are shorter than they are in the centre. The length of each spine as measured in an average specimen is as follows:—

No. 1. 0·0058 mm.	No. 2. 0·0087 mm.	No. 3. 0·0087 mm.
No. 4. 0·0087 mm.	No. 5. 0·131 mm.	No. 6. 0·016 mm.
No. 7. 0·0174 mm.	No. 8. 0·0174 mm.	No. 9. 0·016 mm.
No. 10. 0·0131 mm.	No. 11. 0·0116 mm.	No. 12. 0·0102 mm.
No. 13. 0·0073 mm.	No. 14. 0·0058 mm.	No. 15. 0·0058 mm.

The eggs are numerous, operculate and measure $0 \cdot 026 \times 0 \cdot 013$ mm.

Discussion

Haplorchis rayii, n.sp., stands nearest to *H. pumilio* Looss, 1896 and *H. milvii* Gohar, 1934 for having common kite host. *H. rayii*, n.sp. stands apart from all the known forms of the genus *Haplorchis* in having no pre-pharynx which has been found a basis for classification in many species of the genus.

H. rayii, n.sp. does not come with *H. milvii* Gohar, 1934 in the shape and size of the body, absence of prepharynx, in not possessing a large number of spines on the gonotyle which are 60–70 in *H. milvii*, and the bigger size of the gonads and eggs. The two constricted chambers of vesicula seminalis in our form are of equal size while in *H. milvii* the second chamber of vesicula seminalis is more than double the size of the other.

In its relationship *H. rayii*, n.sp. stands nearest to *H. pumilio* Looss, 1896. It resembles the latter species in the general topography of the organs and the avian host but differs from it in the size of the body, in possessing a sub-terminal oral sucker, absence of prepharynx, and the complete absence of acetabulum which is rudimentary in *H. pumilio*. *H. rayii*, n.sp. has only fifteen keel-shaped rodlets of different sizes on the gonotyle which are of equal size and many in number arranged haphazardly in *H. pumilio*. It further differs from *H. pumilio* in the size of the testis and the

vesicula seminalis. The testis in *H. rayii*, n.sp. is larger, oval and more in the posterior part of the body. The recepticulum seminis is also not dorsal to the testis but it is obliquely on the right side of the ovary. The pattern of vitellaria in our form is unlike *H. pumilio* which forms a link in groups below the testis and covers the parts of the testis and the ovary in a different way.

H. pumilio Looss, 1896	*H. milvii* Gohar, 1934	*H. rayii*, n.sp.
1. Oral sucker terminal and prepharynx present.	Oral sucker terminal and prepharynx present.	Oral sucker subterminal a no prepharynx.
2. Testis round or oval and centrally located in the posterior part of body.	Testis round, small and centrally located.	Testis oval, large and loca more towards posterior end
3. Ovary circular, ventrally located in the same plane as the testis and partly covered by it.	Ovary globular, small and situated anterior to the testis on the ventral side.	Ovary round or oval, lar ventral and situated on right of the median plane body.
4. Vesicula seminalis situated anterior to the testis in between the testis and the intestinal cæcum on the left dorsal side. The two chambers are of different size.	Vesicula seminalis on the left dorsal side of the testis and the two chambers are of different size.	Vesicula seminalis situa on the left dorsal side of testis with two chambers equal size.
5. Recepticulum seminis dorsal overlapping the testis and visible in lateral view.	Recepticulum seminis dorsal but not overlapping the testis and is situated on the opposite side of the ovary on the same plane.	Recepticulum seminis on left side of the body, a li posterior to the ovary not covering the testis.
6. Presence of a rudimentary ventral sucker and gonotyle surrounded by large number of haphazardly arranged spines.	Absence of ventral sucker and gonotyle surrounded by 60–70 spines.	Absence of ventral suc and gonotyle surrounded 15 keel-shaped spines.

Diagnosis

Genus Haplorchis *Looss, 1899*.—Very small trematodes with more or less oval body. Integument covered with spines. Prepharynx present or absent, œsophagus long. Ovary in front of testis, the latter single and located near posterior end of body. Seminal vesicle in several parts. Vitellaria in posterior part of the body. Uterine coils behind ventro-genital-sucker complex, genital sac slightly to one side; gonotyle bears on its surface chitinous armature. Excretory bladder Y-shaped.

Haplorchis rayii, n.sp.—Oval body 0·797×0·674 mm. Oral sucker, 0·049×0·066 mm. and sub-terminal. No acetabulum. Oesophagus 0·125 mm. long. Testis oval, 0·232 mm. in posterior-most quarter of body. Vesicula seminalis of two equal chambers. Ovary, 0·115×0·108 mm.; recepticulum seminis, 0·043 mm. in diameter on the right side of ovary. Vitellaria dorsally and laterally form scattered irregular groups covering little parts of ovary and testis. Ventro-genital sinus, 0·089×0·102 mm., gonotyle bears 15 keel-shaped spines forming a semi-circular crown. Eggs, 0·026×0·013 mm. and numerous.

Host: *Milvus milvus migrans.*

Location: Intestine.

Locality: Mukteswar (Kumaon, U.P.)

Acknowledgements

I am thankful to Professor H. R. Mehra, M.Sc., Ph.D. (Cantab.), F.N.I., Professor of Zoology, Allahabad University for his constant guidance, encouragement and valuable criticism. I am also indebted to Dr. H. N. Ray of Indian Veterinary Research Institute, Mukteswar in whose honour the parasite is named for providing research facilities at Mukteswar.

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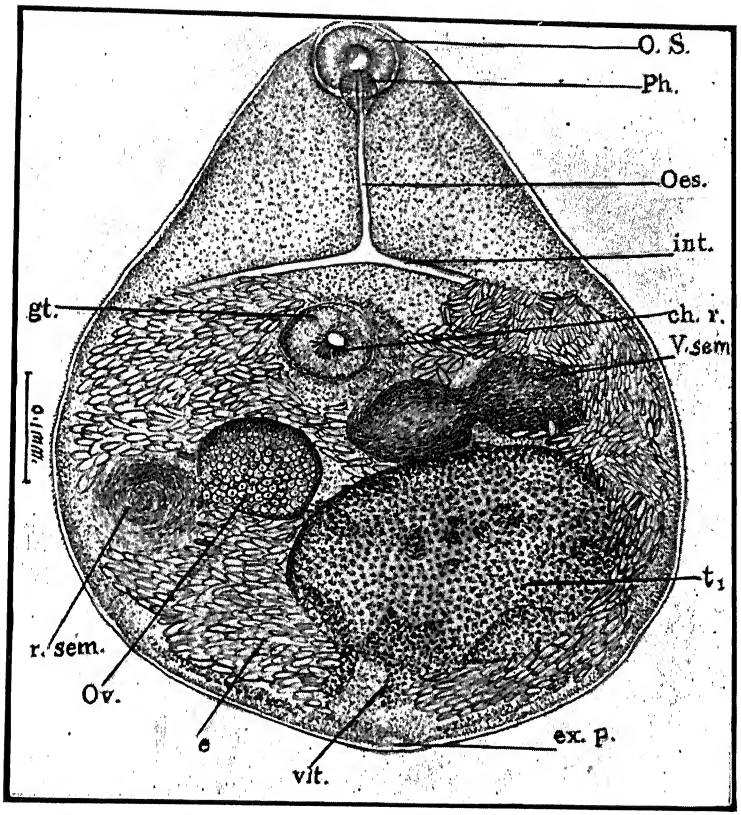
EXPLANATION OF FIGURES

PLATE I. Ventral view of *Haplorchis rayii*, n.sp. (Camera lucida diagram).

PLATE II. Photomicrograph of the posterior part of *Haplorchis rayii*, n.sp.

ch. r., Chitinous rodlets; *e.*, egg; *ex. p.*, excretory pore; *gt.*, gonotyle; *int.*, intestine; *O.S.*, oral sucker; *Ov.*, ovary; *Ph.*, pharnyx; *r. sem.*, recepticulum seminis; *t.*, testis; *v. sem.*, vesicula seminis; *vit.*, vitellaria.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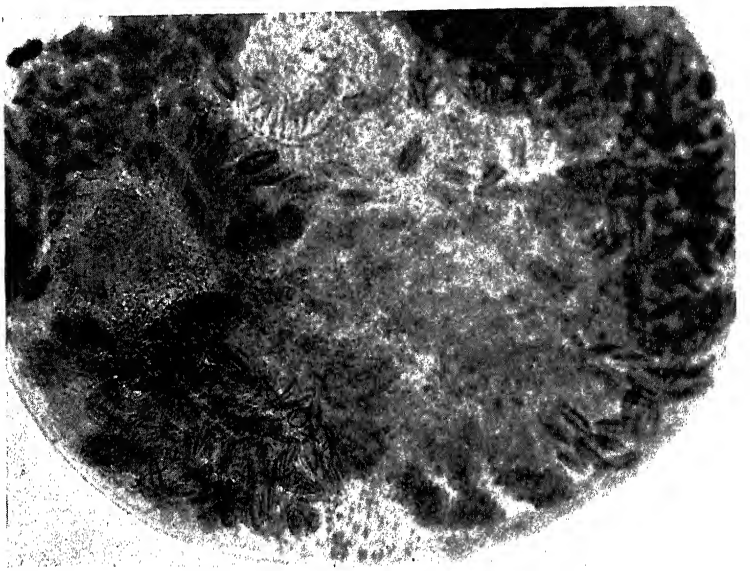

STUDIES ON THE SKULL OF SILUROID FISHES: *CLARIAS BATRACHUS*

Skull of *Clarias batrachus*

By Anjni Kumar

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Historical Account

The exact study of the Cranial osteology of fishes dates as far back as 1873 when Parker described exhaustively the development of the Cranium in Salmon. But the Osteology of Siluroids has not received much attention from the morphologists. Mc. Murrich (1884) described for the first time the skull of American Catfish, *Amiurus catus* in 1884. No further contribution was made in this field until 1919 when James Kindred corrected the mistakes of Mc. Murrich as regards the origin and derivations of different bones. Not only did he describe the adult cranial osteology, but also undertook great pains to analyse the bones in terms of their developmental relations. Bhimachar (1932) studied the Cranial osteology of eight Indian species of Catfishes. David (1935) and Poll (1942) are the recent workers on Clariid skulls.

The cranial osteology of *Clarias batrachus* has been studied by me and many interesting points have been noted in its organisation which may be correlated with the habit of the fish.

Material and Method

The fish was obtained from the ponds of Allahabad, Banaras and Patna. The heads were dissected out and the skulls were prepared by removing the muscles. They were fully dried and finally bleached in 1% hydrogen peroxide to make them white.

General Features of the Skull

The dorsal surface of the skull is very much flattened to produce a ' Cephalic shield ' (Fig. 1). The bones of the skull are completely ossified. There are two fontanelles in the dorsal median line; the anterior fontanelle (*Ant. fon.*) is situated in the frontal region and is normally covered over by a thin piece of cartilage. The posterior fontanelle (*Post. fon.*) is situated in the centre of the supra-occipital and is very superficial and small (Figs. 1 and 3).

The anterior vertebræ are firmly fused to the base of the skull. The post-temporal, a bone of the shoulder girdle has been utilised in the formation of the posterior wall of the skull. The ventral side of the cranium shows a well formed, straight, para-sphenoid extending right from the basi-occipital region to the vomer. Just above the trigeminofacialis foramen, a characteristic teleostean para-sphenoidal wings are developed. The hyo-mandibular is very massive and is firmly fused along its posteriolateral margin. The preoperculum is firmly fused to it. The Entopterygoid is entirely absent. Both the premaxillæ and the vomer are provided with finely curved teeth. The skull is of the platybasic pattern because the cavum cranii clearly extends upto the ethmoidal region.

I. The Ethmoidal region

1. *The Supraethmoid* (Fig. 1 *Se.*)—is an unpaired bone whose anterior end is bifurcated and forms two cornuæ, while posteriorly, it forms the anterior boundary of the anterior fontanelle. The premaxillæ articulate by means of fibrous tissues with the anterior horns of the supraethmoid. While ventrally, the supraethmoid extends to meet the vomer, posteriorly it articulates with the frontals. Laterally it forms the boundary line of the nasal fossa and meets the ectoethmoid.

2. *The Ectoethmoids* (Fig. 1 *Ect.*).—The ectoethmoids are a pair of bones situated on either side of the supraethmoid. Each ectoethmoid tapers anteriorly and articulates with the triangular nasals by fibrous tissues. Laterally, the bone gives out an ectoethmoidal process (Fig. 4 Ect. pr.) which in conjunction with the lacrymal, forms the anterior boundary of the orbit. It is also joined for some distance to the supra-orbital. Dorsally, while the anterior portions join the supraethmoid; ventrally it joins the vomer. The bone is perforated for the passage of the branch of opthalmicus superficialis trigemini.

3. *The Nasals* (Figs.1 and 3 *Na.*).—These are two flat triangular isolated bones abutting against the anterior end of the ectoethmoid.

4. *The Vomer* (Fig. 4 *vo.*)—is a prominent bone on the ventral spide of the ethmoid region. It is an arch-shaped bone. The anterior curvature is beset with minute vomerine teeth. It interdigitates anteriorly with the premaxillæ and the supraethmoid. Laterally, it meets the process of supra-ethmoid and forms the floor of the nasal foosa. Posteriorly, the bone is drawn out into a fine pointed end which articulates with the parasphenoid. It also meets dorsally the ectopterygoid behind the supraethmoid and the ectoethmoid on either side.

5. *The Frontals* (Figs. 1 and 3 *Fr.*)—are very well developed bones. Each frontal is very irregular in outline. Near the ectoethmoid the frontals of either side enclose the elongated anterior fontanelle, which is crest-shaped and is slightly ridged for some distance. In the centre it is perforated, forming a pit. Posteriorly the two frontals are much ridged and are firmly interdigitated with the supra-occipital bone. Laterally the squamosopterotic and the sphenotic join them. Anteriorly the supraethmoid and the ecto-ethmoid are articulated with them.

6. *The Supraorbitals* (Fig. 1 *Sup. orb.*)—are prominent bones lying on the dorso-lateral surface of the skull in between the ectoethmoids and the sphenotics. They form the posterio-lateral boundary of the prominent orbits. The interdigitations are with the frontals on the sides, with the

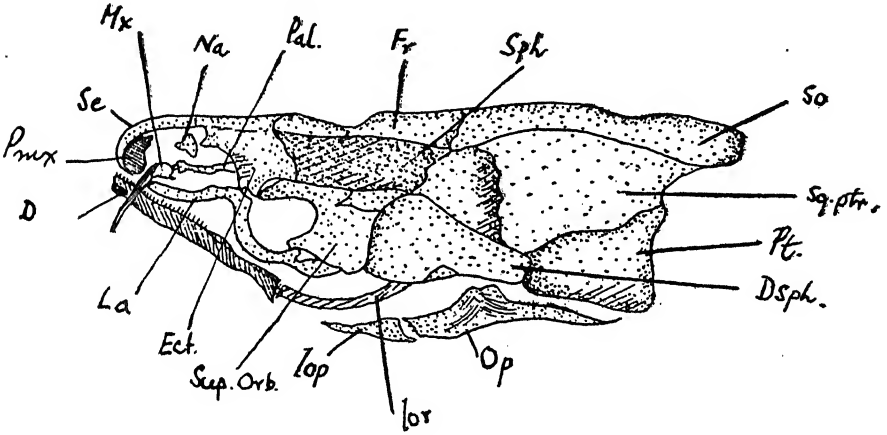

FIG. 2

sphenotics and the dermosphenotics posteriorly. While laterally the bones of the circumorbital series are attached to them through the infraorbitals.

7. *The Sphenotics* (Figs. 1 and 3 *Sph.*).—These are a pair of bones, each situated behind the frontal of its own side on the lateral side of the cranium. Each sphenotic is bounded anteriorly by the frontals and the supraorbitals, posteriorly by the dermosphenotic and the squamosopterotic. There is a definite groove on the cerebral side of the bone for lodging a portion of the semi-circular canal. Ventrally it is strongly ridged for the support of the hyomandibular muscles. The trigmenial foramen is also bounded by this bone. It articulates with the alisphenoid ventrally and posteriorly with the pro-otic.

8. *The Dermosphenotics* (Figs. 1 and 3 *Dsph.*)—are two conspicuous bones occurring in the lateral part of the Cranium below the supra-orbital,

Each bone is roughly triangular in shape. Anteriorly, it is bounded by the supra-orbital. Posteriorly, it is firmly interdigitated with the squamoso-pteriotic and the post-temporal. Dorsally it is grooved near the operculum for the attachment of muscles supporting the opercular apparatus.

9. *The Orbitosphenoid* (Fig. 4 *Os.*)—is a single bone lying in the ventro-lateral side of the skull forming the side wall of the Cranial cavity situated between the ectoethmoid and the trigeminal foramen. Posteriorly, it articulates with the alisphenoid and in conjunction with it, forms the boundary wall of the trigeminal foramen. The dorsal surface of the bone is notched for the attachment of the pterygoid muscles. Ventrally it articulates with the parasphenoidal axis. And it is firmly interdigitated with the frontals above.

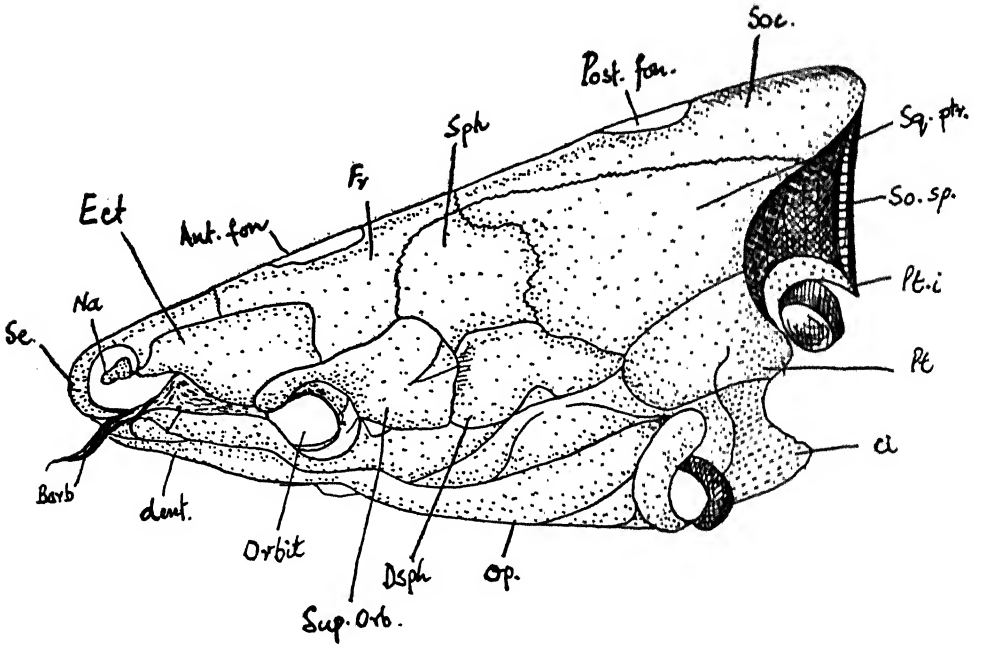

FIG. 3

10. *The Suprasphenoid* (Fig. 4 *Ss.*)—is an unpaired bone which is completely fused with the parasphenoid throughout its length between the foramina for opthalmic (V) and trigeminal (VII) nerves. Posteriorly, the bones join the pro-otics.

11. *The Alisphenoids* (Fig. 4 *Alis.*)—are flat bones occurring between trigeminal and optic formina forming the boundary of these two foramina. Posteriorly the bone extends to the point where the hyomandibular and the

sphenotic articulate with each other. Anteriorly, it articulates with the orbitosphenoid. Dorsally, it firmly joins with the frontals and the sphenotics. Laterally, it touches the parasphenoidal spur.

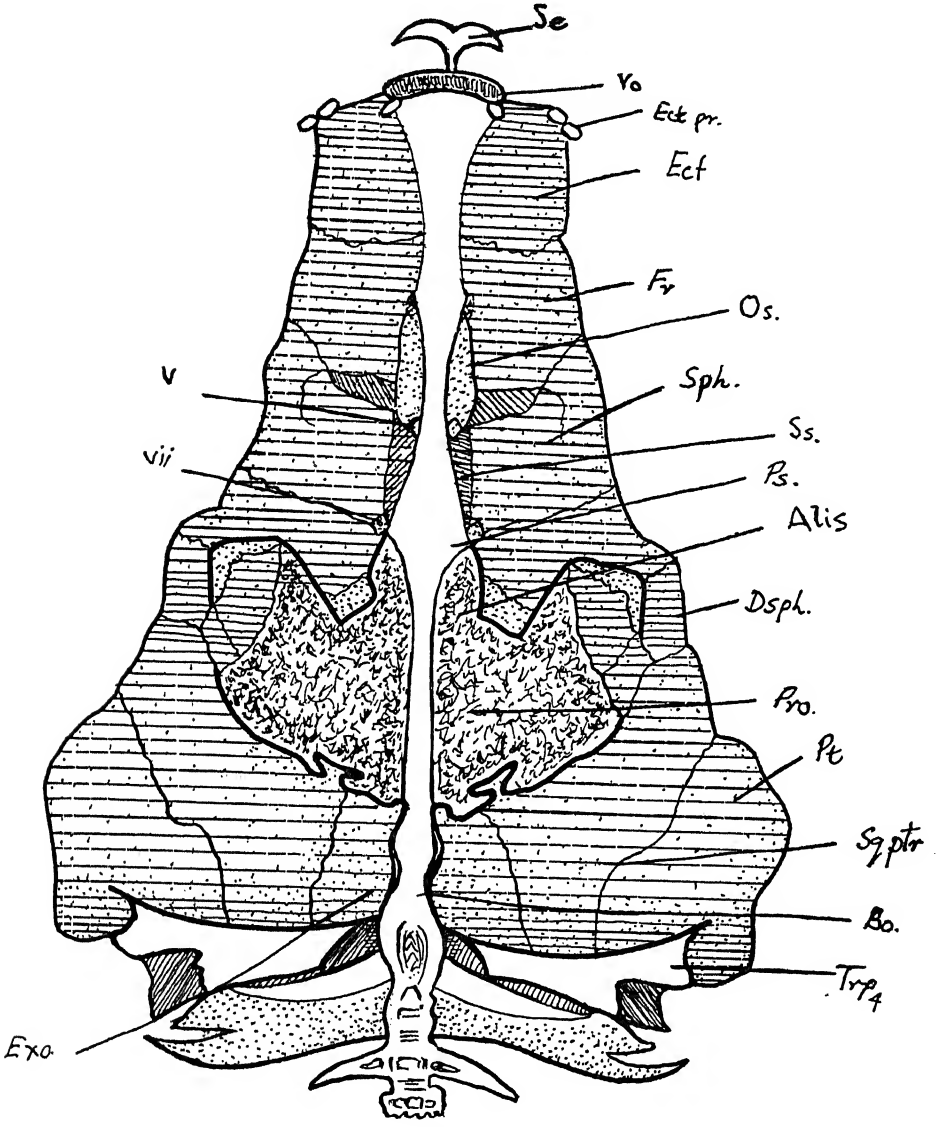

FIG. 4

12. *The Parasphenoid* (Fig. 4 *Ps.*)—is the longest bone of the skull extending from the basioccipital to the vomer on the ventral side and forms the floor of the cranium. Near the trigeminial foramen, it give out two

lateral bony spurs which form the characteristic wings of the teleostean parasphenoid. The alisphenoids articulate along these on the lower side. It articulates with the suprasphenoid. In the ethmoidal region, it meets the orbitosphenoid and the suprasphenoid and the vomer.

13. *The Squamosopterotics* (Fi . 1 *sq. pt.*).—These two bones form the posterior edges of the cranium. Each one is an irregular bone occurring in the posterior region of the cranium between the supra-occipital, the sphenotic and the post-temporal. Laterally, it is bounded by the dermo-sphenotic. In conjunction with the sphenotic it forms an articulating facet for the hyomandibular. There is a cavity in its lower portion which forms the recess for the lateral semi-circular canal. The bone articulates anteriorly with the sphenotic. Ventrally, with the pro-otic and mesially with the post-temporal by numerous spicules.

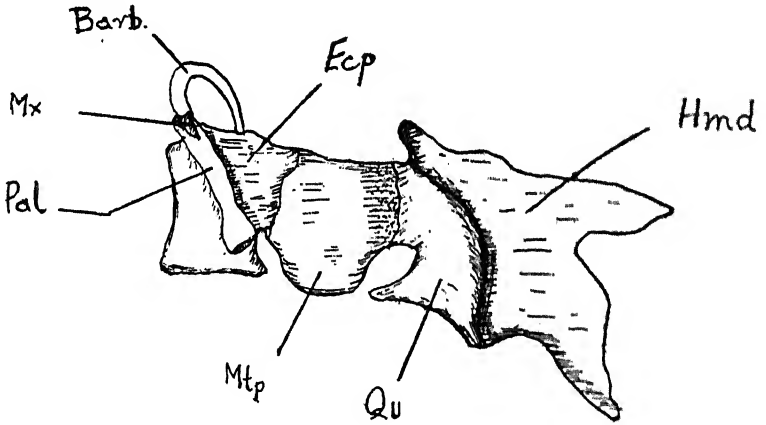

FIG. 5

14. *The pro-otics* (Fig. 4 *Pro.*).—They are smooth, thin square-shaped bones lying in the lateral surface of the brain case between the trigmenial foramen and the ex-occipital. In the middle, there is a slight elevation for the attachment of the hymandibular muscles. At the posterior side, there occurs a recess which is termed the recessus sacculorum. Similarly in the middle region of the pro-otic there is a well developed vertical ridge which produces another groove for lodging the anterior semi-circular canal. The recessus utriculi is marked out at the posterior extremity as indicated by the presence of another small ridge. However, there is no trace of the eye muscle canal which is present in other teleosts.

Anteriorly, the pro-otic articulates with the supra-sphenoid and the parasphenoid. Posteriorly, it is bounded by the basioccipital; laterally it

is joined to the squamosopterotic. It is separated from the epiotics by a cartilaginous strip. Dorsally, the sphenotic and the squamosopterotics are firmly joined to it while the alisphenoid just touches it. Posteriorly, it is firmly articulated with the exoccipital.

15. *The Epiotics* (Fig. 4 *Epo.*).—Lying close to the ex-occipital at the posterio-lateral extremity of the skull, are the two epiotics. Each bone is roughly polygonal in shape. It has a convex outer side and a plain cerebral sides. In the centre of the bone, there is a permanent partition dividing it into two parts for the enclosure of the semi-circular canals. In the centre, there is a permanent foramen for the exit of vagus nerve. It articulates with the supra-occipital above and the ex-occipital below.

16. *The Supra-occipital* (Figs. 1 and 3 *Soc.*).—It is a broad single bone forming the roof of the occipital region. The outer surface of the bone is finely granulated. In the centre of the bone on the dorsal side a fontanellæ is situated which is covered by a cartilaginous strip, the removal of which

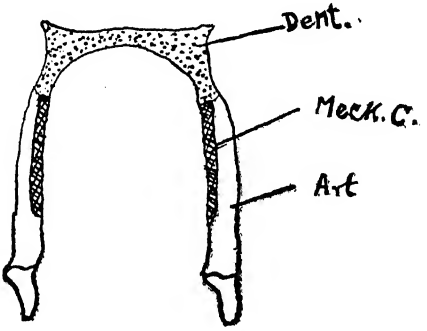

FIG. 6

exposes a groove and a perforation in the centre; the latter is the posterior fontanellæ proper. The shape of the bone is roughly rhomboidal. Anteriorly it thins down to a narrow point to meet the frontals, while posteriorly it has a convex shape and is much broader. A thick downward occipital spine (Fig. 3 *So. sp.*) is given out posteriorly which serves to connect the skull with the anterior vertebræ, through the attachment of muscles. Dorsally, it articulates with the frontals in front, the squamosopteriotic along the sides and posteriorly it forms the boundary of the ex-occipitals. But it does not take part in the formation of foramen magnum. Ventrally, it is joined to the basioccipital.

17. *The ex-occipital* (Fig. 4 *Exo.*)—forms the complete boundary of the foramen magnum. The two bones on each side fuse dorsally in the

middle line and thus exclude the supra-occipital from meeting the foramen magnum. On the lower side, they form the base of the cavum cranii and the roof of the sinus imparis. Laterally, in continuation with the supra-occipital ridge they form the posterior boundary of the otic recess. Laterally, again, there occurs foramina for the passage of hypoglossal and the vagus nerves. The Ossification is incomplete in this region and hence much of cartilage remains between the articulating surfaces.

18. *The Basioccipital* (Fig. 4 *Bo.*)—forms the posterior most element of the cranium on the ventral surface. Anteriorly, the bone tapers to a fine point for articulation with the parasphenoid. The hinder portion is quite thick and concave in shape. The lower surface of the bone is prominently grooved which forms the floor of the recessus sacculi.

The articulations are with the parasphenoid anteriorly and laterally, with the pro-otics and the ex-occipital.

The supratemporals and the parietals are absent.

19. *The Post Temporal* (Figs. 1, 2 and 3 *PE.*)—which is originally a bone of the shoulder girdle has become a part of the cranium. Dorsally, it is completely fused along its lateral side with the squamosopterotic. Its upper surface is flattened. On the lower side it gives an out growth which joins the elongated and expanded transverse process of the fourth vertebræ which covers the air bladder. Between these inferior limbs of this bone a considerable cartilage persists and wherein the articulating surfaces of the cleithrum is fitted.

II. The Temporal and the Opercular Bones

The post temporals and the supratemporals have already been described.

20. Sub-temporals are not clearly seen and are probably absent.

21. The preoperculum is a long and thick bone firmly articulated on the outer side of the hyomandibular and the quadrate. It has got a foramen for the passage of the hyomandibular nerve. Anteriorly the articular bone touches it while posteriorly it is bounded by the prominent knob of the hyomandibular.

III. The Circumorbital Series

22. The Infraorbitals (Fig. 1 *Ior.*).—Extending from the supra-orbitals towards the lacrymal, three small infra-orbitals form a complete chain o' bones which form the lower boundary of the orbit. There is no firm inter-digitation between them. They are held together by fibrous tissues and

facial muscles of the orbital region. The first infra-orbital bone is broadened towards the post-orbital side and its surface is very much granulated. The other end tapers to a point. The next infra-orbital bone is rather small and slender. The third infra-orbital bone again gets thickened on the anterior side.

23. *The Lacrymals* (Fig. 2 *La.*)—belong to the infraorbital series and join the last infra-orbitals. It is a splint-like bone and extends towards the maxilla. There is a good deal of cartilage at the point where it joins the supraethmoids. It forms the outer boundary of the nasal chamber.

24. *The Nasals* (Figs. 1 and 3 *Na.*)—are flat, triangular bones abutting the anterior end of the ectoethmoid between the two sides of the supra-ethmoid. There is no proper ossification to the cranial surface and they are only held by fibrous tissue.

IV. The Maxillary Series

25. *The Pre-maxillaries* (Fig. 2 *Pmx.*)—are the anteriormost bones of the cranium on the ventral side. Each one is a rectangular piece of bone in which two areas are discernible. The upper portion on the ventral side is provided with 9 rows of pointed conical teeth fitted in sockets. The smaller lower portion is quite smooth and edentulous and hangs freely in the nasal chamber. The bone is grooved for the supraethmoidal cornuæ. The two bones are united in the centre just below the notched end. Dorso-aterally, the bones are joined to the palatines by fibrous tissues.

26. *The Maxillaries* (Fig. 2 *Mx.*).—Each maxillary bone is devoid of teeth and is very much reduced in size. It has much shifted from its original place and projects at right angles from the general surface of the cranium. Posteriorly it is thickened and knobed while the middle part is quite thick. The anterior surface is notched for the attachment of the cartilaginous core of the maxillary barbel (Fig. 2 *Barb.*).

It is firmly articulated with the palatine and any movement of the latter moves this bone. The attachment with the pre-maxillary is very feeble.

V. The Palatoquadrate and The Hyomandibular Series

27. *The Palatines* (Fig. 2 *Pal.*).—Each palatine is a long, rod-like bone, much flattened at the posterior end. In the middle of the bone there is a prominent knob which fits in the socket formed by the ectoethmoid bone. The anterior part is grooved for the attachment of the maxillary knob. Anteriorly, it is very feebly attached to the pre-maxillary. A considerable amount of cartilage persists at both the ends of the bone.

28. *The Ectopterygoid* (Fig. 5 *Ecp.*).—Lying just behind the vomer are crescent-shaped ectopterygoids. Posteriorly, each bone is attached to the anterior flat end of the metapterygoid. The outer free end of the bone is pointed and the muscles binding the palatines are attached to its posterior face.

29. *The Metapterygoids* (Fig. 5 *Mtp.*).—Each bone is a small, flattened bone whose anterior broad surface articulates with the ectopterygoid. The middle region is slender. The posterior end is attached to the quadrate.

30. *The Quadrates* (Fig. 5 *Qu.*).—It is a triangular bone lying in between the hyomandibular and the metapterygoid. Posteriorly, it bounds the foramen from which the hyomandibular nerve emerges. The cartillaginous symplectic lies between the hyomandibular and the preoperculum. Anteriorly it is grooved, forming a facet for articulation with the lower jaw.

The articulations of the quadrate are with the metapterygoid and articular anteriorly, with the hyomandibular posteriorly, and with the preoperculum anteriolaterally.

31. *The Hyomandibular* (Fig. 5 *Hmd.*)—is a flat, irregular massive bone. Laterally, it is provided with a well developed knob through which it articulates with the operculum. Posteriorly, its flattened surface fits in a groove formed in the sphenotic and the squamosopterotic and thus suspends the platoquadrate arch to the cranium. The general surface on both the sides is deeply notched for giving attachment to the adductor muscle. Laterally, it is attached to the preoperculum along its entire length. It is not connected with the parasphenoidal axis.

VI. The Mandibular Series

Each mandible consists of the articular and the dentary held together anteriorly by fibrous tissues. Posteriorly, they fit into grooves formed by the quadrate.

32. *The dentary* (Fig. 6 *Dent.*)—is the longer anterior bone o ' the series. Anteriorly, on the lower side it is provided with 10 to 15 rows of conical teeth. The two dentaries are attached by ligament at the symphysis, while posteriorly it meets the articular. The broad posterior surface of it is feebly grooved for the Meckel's cartilage.

33. *The Articular* (Fig. 6 *Art.*)—is a thick stumpy bone. Posteriorly it has a well developed articulating surface for the quadrate.

VII. The Opercular Series

34. *The Operculum* (Fig. 26 *op.*)—is a roughly triangular bone. It articulates with the knob of the hyomandibular by a concave articulating

surface. Its posterior pointed end is free, while the anterior end is blunt and is attached to the inter-operculum by a ligament.

35. *The inter-operculum* (Fig. 2 *Iop.*)—is a laminar bone attached to the operculum by a ligament posteriorly. Anteriorly, it is attached to the upper surface of the epithyal.

Discussion

In *Clarias batrachus*, the various bones covering the dorsal surface of the brain are very much spread out in their broad dimensions and firmly fused together resulting in the formation of the characteristic cephalic shield of the siluroids. A similar cranial shield has been described in *Ophiodon* and other *Loricati* by Gutberet (1916). Huxley got confused by such resemblances and he grouped Clariinæ and Loricariidæ together while classifying the fishes of the Devonian Era. Woodward has described a similar shield in *Dactylopterus* and other fossil fishes of the families Cephalaspidæ and Cocosteidæ. A careful examination of the component bones in these diverse forms, however, reveals that it is only a superficial resemblance due to convergence in evolution rather than due to any genetic relationship.

In some siluroid, *viz.*, *Eutropiichtyphys vacha* and *Pseudeutropius garua* there is no well developed cranial shield but on the other hand the component bones are very thin and variously ridged. The presence or absence of a well developed cephalic shield in the siluroids may be explained in relation to the mode of life of the fish. In sluggish and sedentary siluroids, like *Clarias*, the shield has a protective value, while in active swimmers like *Eutropiichthys* such a disposition of bones might have proved a constant hindrance in gaining the speed.

The other point of general interest in the morphology, of the cranium is the occurrence of a well developed supra-occipital spine. In *Clarias batrachus*, spina occipitis forms a well developed shelf all along the posterior occipital region for the attachment of the muscles which bring about a cohesion of the cranium and the vertebral column. A median longitudinal section of the skull of *Clarias batrachus* clearly shows that the cranial cavity passes into the orbitosphenoid and the ethomoid regions. Hence the skull of Clarias is organised on the primitive platybasic pattern as observed in the lower archaic Teleostean fishes like Ganoids, etc. The occurrence of a toothed vomer is another primitive feature observed in *Clarias batrachus* because Bhimachar has shown that small edentulous condition of vomer is met in higher siluroids like *Arius*, etc.

Unlike other teleosts, here the frontals interdigitate firmly and with the supra-occipital posteriorly. The parietals have been found to be absent in Clarias like that of other siluroids.

The orbitosphenoid in *Clarias batrachus* is elongated and strongly developed. This primitive feature of the bone is probably retained by *Clarias* for giving support to the well developed Cranial surface. This seems to be specially so in the case of other sedentary siluroids also.

Tate Regan (1911) has reported the absence of suprasphenoid in Siluroids. Bhimachar (1933), however, found this bone to be present in eight Indian Siluroids studied by him. In *Clarias batrachus* this bone has been found to be completely fused with the parasphenoid along with its lateral margins between the foramina for V and VII nerves. The presence of characteristic teleostean wings of parasphenoid shows that this fish has an affinity with the lower teleostean fishes because it is not so prominent in higher Siluroids like *Amiurus catus*.

The presence of dermosphenotic bone in *Clarias* can be explained due to the formation of the cephalic shield, Goodrich designated this bone as the lateral checkbone or possibly a modified preopercular. But as the preoperculum has been found to be fused along the outer border, of the hyomandibular the term dermosphenotic (Gregory) has been adopted here.

The Squamosopterotic and the pro-otic are very well developed in *Clarias* and both the bones enclose the internal ear. There is no trace of eye muscle canal or myodome. There is no supratemporal present in the case of *Clarias batrachus* though its presence has been observed by Bhimachar in other Indian Siluroids.

The nasal and the infra-orbital bones are very ill developed in *Clarias*. The maxillary and the palatines are firmly fused together so that the movement of the one effects that of the other. The maxillary is completely edentulous and is concerned only with providing attachment to the maxillary barbel. Distinct ectopterygoid has been observed in *Clarias* though it has a splint-like appearance and is not very strongly developed. Hence, there seems to be no justification for Kingsleys' statement that in all Siluroids there is only one pterygoid—the metapterygoid.

The present study of *Clarias batrachus* skull, thus clearly shows that the fish has retained many primitive features of the lower teleostean group and also that it shows certain specialised osteological features which have developed due to the special habit and habitat of the fish.

ACKNOWLEDGEMENTS

I am deeply indebted to Prof. D. R. Bhattacharya for his suggestion of this problem to me and for his constant help and valuable guidance throughout the course of this work. I am also very grateful to Dr. S. K. Dutta for his kind advice and the pains he took in seeing the manuscript. My thanks are also due to Dr. H. R. Mehra for giving me departmental facilities and Dr. S. L. Hora, Director of Zoological Survey, Calcutta for giving me library and numerous other facilities.

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EXPLANATION OF FIGURES

FIGS. 1–6. *Clarias batrachus*. Fig. 1. Dorsal view of the skull. Fig. 2. Side view of the skull to show the anterior bones. Fig. 3. Lateral view of the skull. Fig. 4. Ventral view of the Cranium. Fig. 5. The maxillary and the palatoquadrate bar. Fig. 6. The lower jaw.

EXPLANATION OF ABBREVIATIONS

Alis.	..	Alisphenoid.	*Pmx.*	..	Premaxilla
Ant. font.	..	Anterior fontanelle	*Pt.*	..	Post-temporal
Art.	..	Articular	*Pti.*	..	Inferior limb of Post-temporal
Barb.	..	Barbel			
Bo.	..	Basioccipital	*Pro.*	..	Pro-otic
Cl.	..	Cleithrum	*Ps.*	..	Parasphenoid
Dent.	..	Dentary	*Qu.*	..	Quadrate
Ecp.	..	Ectopterygoid	*Se.*	..	Supraethmoid
Epo.	..	Epiotic	*So. Sp.*	..	Supra-occipital Spine
Ex.	..	Exoccipital	*Soc.*	..	Supra-occipital
Fr.	..	Frontal	*Sph.*	..	Sphenotic
Hmd.	..	Hyomandibular	*SS.*	..	Suprasphenoid
Iop.	..	Interoperculum	*Sq. ptr.*	..	Squamosopterotic
Ior.	..	Infraorbital	*Sup. orb.*	..	Supra-orbital
Meck. C.	..	Meckel's Cartilage	*Tr. Pr$_4$.*	..	Transverse process of IVth Vertebra
Mpt.	..	Metapterygoid			
Mx.	..	Maxillary	*Vo.*	..	Vomer
Na.	..	Nasal	*V.*	..	Foramina for opthalmic nerve
Op.	..	Operculum			
Os.	..	Orbitosphenoid	*VII.*	..	Foramina for trigmenial nerve
Pal.	..	Palatine			
Post. font.	..	Posterior fontanelle			

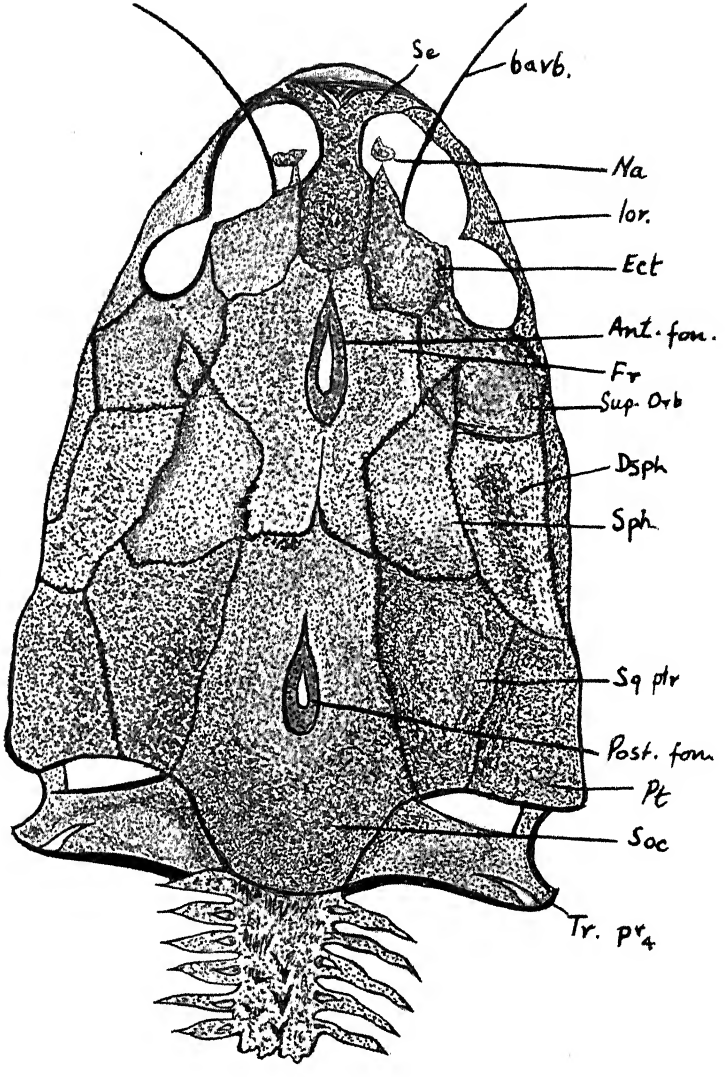
Se
barb.
Na
lor.
Ect
Ant. fon.
Fr
Sup. Orb
Dsph
Sph
Sq ptr
Post. fon.
Pt
Soc
Tr. pr4

FIG. 1

A NOTE ON DIURNAL VARIATIONS OF ACIDITY AND RESPIRATORY PROCESSES IN THE SUCCULENT LEAVES OF *COLEUS AROMATICUS*

By S. Ranjan and B. S. Bisht

(*Botany Department, University of Allahabad*)

The succulent leaves of *Coleus aromaticus* were examined for total titratable acidity, pH content, and respiratory processes under natural conditions. It was investigated that they show Crassulacean type of acid metabolism (Bennet Clark, 1933), *i.e.*, the acidity increased during night or in darkness and decreased with the return of day-light or, under illumination, the accumulated acids disappeared. In early July, *i.e.*, at the beginning of the rainy season, the stem cuttings were propagated; the leaves started to gain in thickness and became juicy and fleshy in about 2 months time. The experiments were carried out in the months of November and December 1953. An attempt was also made to identify the organic acids involved by the method of Paper Partition Chromatography. The findings showed that the pivotal acid was malic which showed considerable loss and gain. Respiratory studies were done by Warburg's Constant Volume Type Respirometer at 28° C. The graphic representation of the titrable acidity curve as noted against time (Fig. 1) clearly shows that normal alternating periods of light and darkness are responsible for diurnal periodicity of acid contents in the fleshy leaves of *C. aromaticus*. Total titrable acidity is represented by N/25 NaOH required to neutralise the acids present in the extract obtained from 1 gm. of fresh leaf material. Leaves collected in the morning at 5 A.M. recorded the maximum acidity of 5·09. Under the influence of day-light the accumulated acids decomposed and the lowest value of 0·56 was estimated at 4 P.M. As soon as dusk set in, the acids started to accumulate. The pH content (Fig. 2) was inversely correlated with titratable acidity; indicating thereby that acidity whether measured as pH or by T.T. acidity, undergoes wide variations.

The respiratory rate (Fig. 3) as measured by O_2 uptake showed no relationship with T.T. acidity. It is generally assumed that acid metabolism has no effect upon O_2 uptake. The R.Q. curve (Fig. 4) also shows marked fluctuation. It reached as low as 0·33 at 7 P.M. and 10 P.M. The highest R.Q. of 1·6 was measured at 12 Noon. It shows a tendency to rise above unity in day-light. With the advent of darkness, it falls below unity. It is

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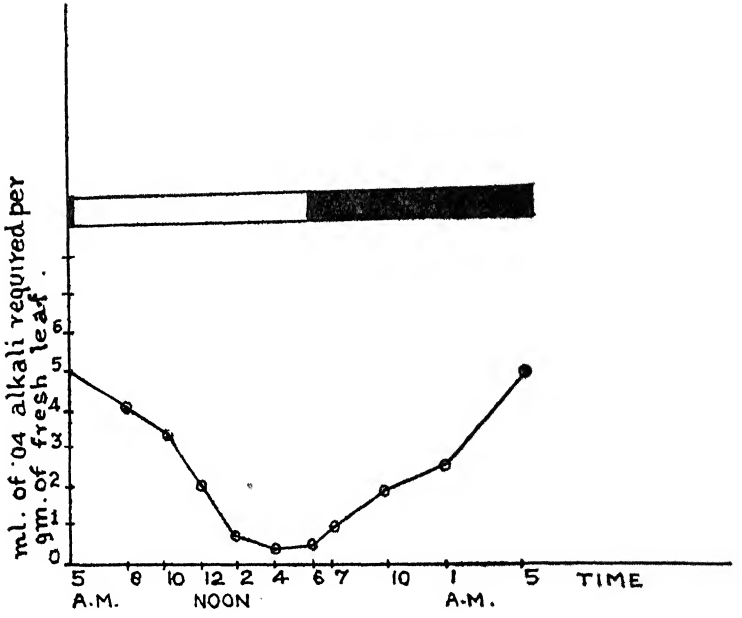

FIG. 1. Diurnal Variation in Titratable Acid of *Coleus aromaticus* Leaves.

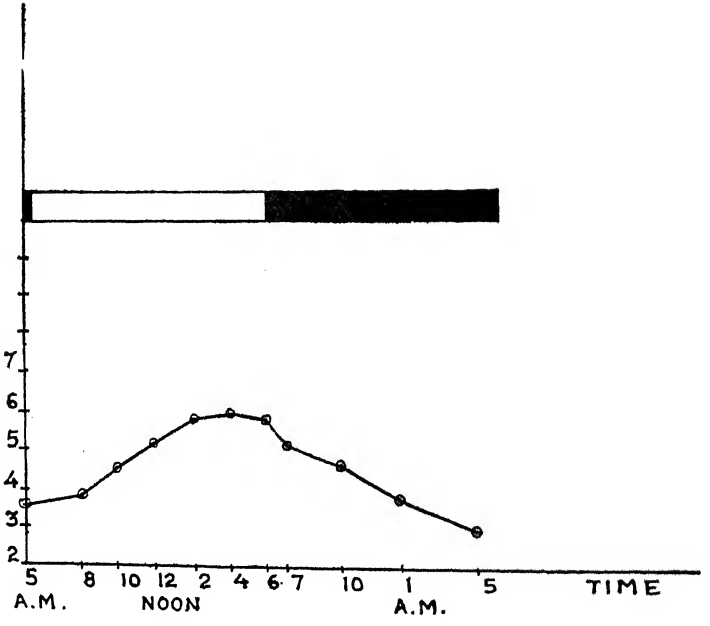

FIG. 2. Diurnal Variation in pH Content of *Coleus aromaticus* Leaves.

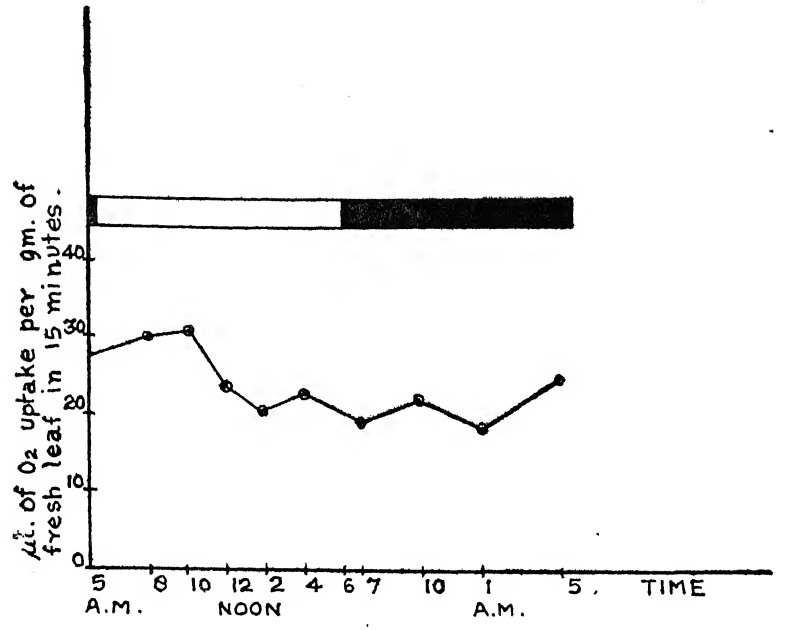

FIG. 3. Respiration Rate of *Coleus aromaticus* Leaves.

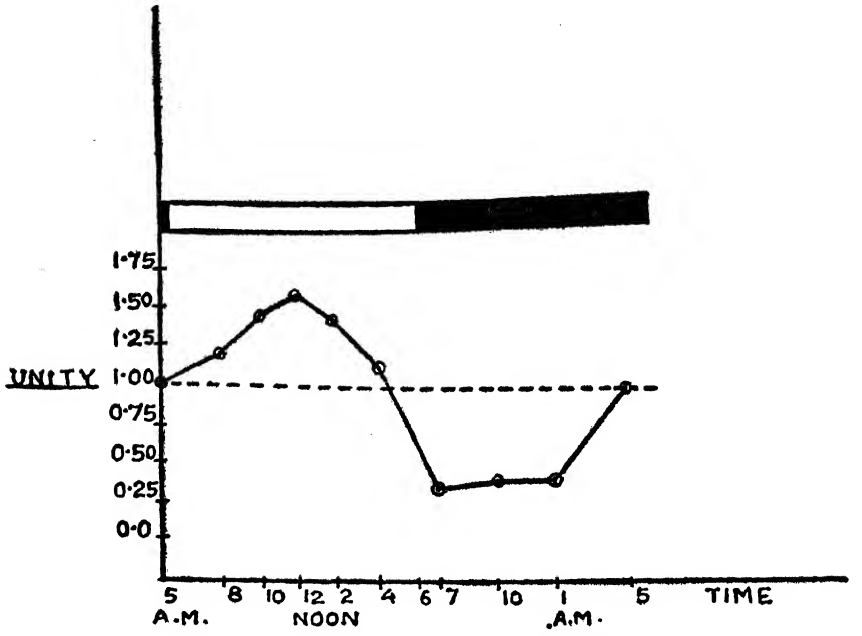

FIG 4. Diurnal Variation in R.Q. of *Coleus aromaticus* Leaves.

only during the accumulation of acids, the R.Q. value is lower than unity; this phenomenon is peculiar to succulent plants (Bennet-Clark, 1933; Pucher *et.al.*, 1947 and Thomas, 1949). Immediately after darkening or dusk the CO_2 output is low from leaves, it can reasonably be said that the normal respiratory CO_2 is being utilized for the production of acids. The demonstration of R.Q. of unity, when acid accumulation is at its maximum, provides explanation that the acid-accumulating power of the leaf is exhausted and the normal respiratory CO_2 is freely given out. With the return of daylight the accumulated acids are decomposed and CO_2 entrapped during the formation of acids in darkness comes out in excess of normal respiratory CO_2, with the result R.Q. rise above unity.

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EFFECT OF SULPHA-DRUGS ON THE GROWTH OF *PESTALOTIA* SPP.

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Introduction

Sulpha-drugs are a group of chemotherapeutic agents capable of curing serious generalized or systemic bacterial infections in man. In 1935, Domagk, a German investigator first observed the clinical value of prontosil, a red compound derived from azo-dyes. *Para*-amino-benzene-sulphonamide, was shown to be the effective principle of the prontosil molecule, and this substance was named Sulphanilamide. It was first of the group to receive wide clinical trials. The marvellous therapeutic results obtained in various infections have naturally stimulated extensive pharmacological and bacteriological investigation on these compounds.

Such a widespread use of Sulpha-drugs against the infection of bacterial origin suggested the possibility of using them for the treatment of mycotic infections also. Probably there is no bacterial or fungal infection in men against which one or more of the Sulpha-drugs have not been tried. In plant pathology, however, the use of these drugs has been very restricted.

Fourneau *et al.* (1936), first investigated the effect of Sulphonamides on fungi and they reported that the growth of *Aspergillus niger* was greatly delayed by the addition of Sulphanilamide to the culture medium. Lewis and Hopper (1941), Dimond and Thompson (1942), and Senturia and Wolf (1945), confirmed the fungi-static action of these drugs on some other fungi also.

It was, therefore, considered desirable to study their effect on *Pestalotia* spp.

Material and Methods

The two species used in the present investigation were *Pestalotia malorum* and *Pestalotia psidii* isolated from rotten apples and mummified guavas respectively.

Asthana and Hawker's medium A* without Agar was used as Control. In order to study the action of Sulpha-drugs, they were added to it individually in the following concentrations:—0·025%, 0·05%, 0·075% and

* It contains, Glucose 5 gm., KNO_3, 3·5 gm., KH_2PO_4, 1·75 gm., $MgSO_4.7H_2O$, 0·75 gm. and water 1000 c.c.

0·1%. Throughout the investigation only Pyrex glasswares, double distilled water and as far as possible chemicals of Analytical grade or purest available were used. Each 150 c.c. Erlenmeyer flask containing 50 c.c. of the control medium alone or with the addition of definite quantity of Sulpha-drug was sterilized at 15 lb. pressure for 15 minutes. Four replicates were used for each series and inoculations were made by the Agar disc method as suggested by Garrett (1936). It has already been reported by the authors (1948), that the best growth of these species was obtained at 20° C. The cultures were therefore, incubated at that temperature for 3 weeks. At the end of that period, mycelium and spores of each flask were collected separately on previously dried and weighed Whatman No. 42 filter-papers. They were dried in an oven at 60° C. for 72 hours and were subsequently transferred to a dessicator. Weighing was completed rapidly. The weight obtained was used as a quantitative measure for comparing the growth of the organisms on different solutions of the Sulpha-drugs and the control.

In order to find out whether some morphological changes were brought about by inclusion of Sulpha-drugs to the culture medium some microscopic studies were also undertaken. Ridgway's (1912), "Colour Standards and Nomenclature" was employed for describing the colours.

OBSERVATIONS

The weight of the fungus obtained with different doses of various Sulpha-drugs is given in Table I. Since there was no marked difference between the individual replicates, only the average weights are shown in the table.

Table I shows that *P. psidii* did not always show the same reactions as *P. malorum*. The best growth of *P. psidii* was observed when 0·025% sulphadiazine was added to the control medium. The growth of *P. malorum* on that medium was extremely poor. In every other case except 0·025% sulphadiazine, the dry weight of *P. psidii* was less on media containing the Sulpha-drugs. The decrease in weight as compared with the control was not so pronounced in solutions containing sulphamerazine and Sulphaguanidine but even in these there was marked reduction in weight. Sulphanilamide, sulphathiazole and sulphapyridine, however, appeared unfavourable for the growth of the fungus, as it was observed that even a low percentage of 0·025 considerably inhibited the development of both the species of *Pestalotia*. In every case an increase in the amount of Sulpha-drug in the nutrient media brought about a decrease in the weight of the fungus, and the poorest growth was observed with 0·1% concentrations of the

TABLE I

Growth of Pestalotia *spp. after* 21 *days incubation at* 20° *C. on different concentrations of various Sulpha-drugs*

Sulpha-drug	Concentration in %	Average Yield	
		P. malorum	*P. psidii*
		Gm.	Gm.
1. Sulphadiazine	0·025	0·0076	0·5134
	0·050	0·0057	0·4659
	0·075	0·0054	0·3886
	0·100	0·0048	0·3671
2. Sulphaguanidine	0·025	0·2379	0·2851
	0·050	0·2365	0·2832
	0·075	0·2347	0·2778
	0·100	0·2120	0·2758
3. Sulphamerazine	0·025	0·3524	0·3581
	0·050	0·3461	0·3547
	0·075	0·3359	0·3528
	0·100	0·3315	0·3464
4. Sulphanilamide	0·025	0·0126	0·0085
	0·050	0·0104	0·0078
	0·075	0·0088	0·0069
	0·100	0·0072	0·0066
5. Sulphapyridine	0·025	0·1910	0·1988
	0·050	0·1706	0·1932
	0·075	0·1485	0·1854
	0·100	0·0952	0·1170
6. Sulphathiazole	0·025	0·0095	0·0092
	0·050	0·0083	0·0085
	0·075	0·0037	0·0070
	0·100	0·0026	0·0058
7. Control		0·4924	0·4758

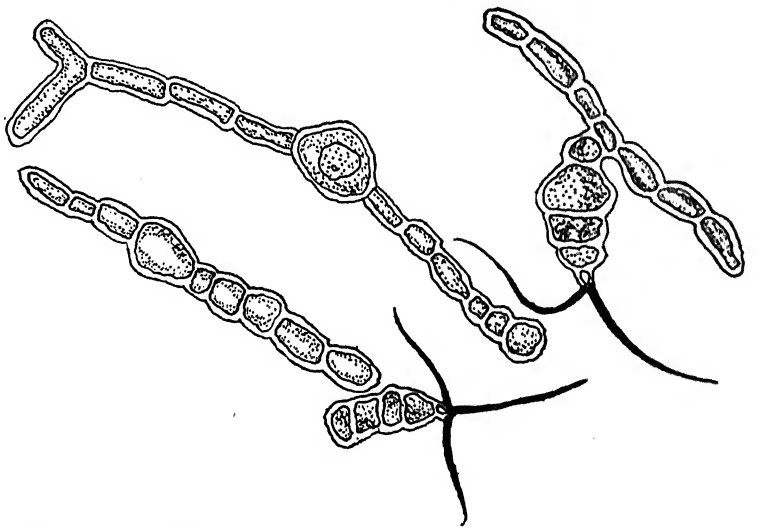
Sulpha-drugs (*Vide* Plates IV to X, Photographs 1–13) where *Pestalotia* sp. 1 refers to *P. malorum* and *Pestalotia* sp. 2 to *P. psidii*.

Microscopic studies revealed the morphological changes that were brought about on account of the addition of different concentrations of various Sulpha-drugs. The results are summarized below:—

A. *Characters of* P. malorum

(*a*) *Medium A* (Used as Control).—Mycelium was branched, septate and it was filled with homogeneous protoplasm. Spores were spindle-shaped with double-walled septa. The anterior and posterior cells were hyaline and the middle ones were auburn-coloured. They were mostly 4-septate and the number of cilia varied from 1 to 4.

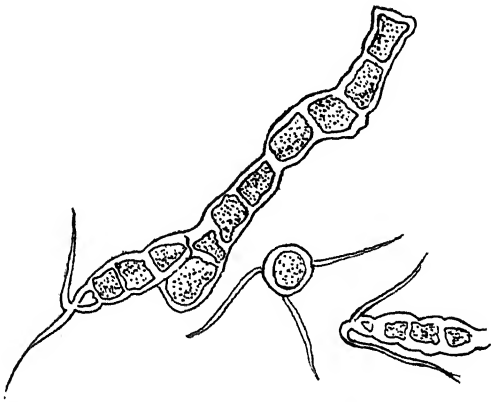
(*b*) *Sulphadiazine*.—The mycelium was closely septate, hyaline or buff yellow in colour. Numerous hyaline or tawny yellow chlamydospores were produced. Also a few spores of peculiar shape were developed. They were mostly 4-septate, clay-coloured with 2-3 cilia. An increase in the concentration of the drug decreased the number of spores, besides this no other change was evident (*Vide* Text-Fig. 1).



TEXT-FIG. 1. Shows hypha, spores and chlamydospores of *P. malorum*.

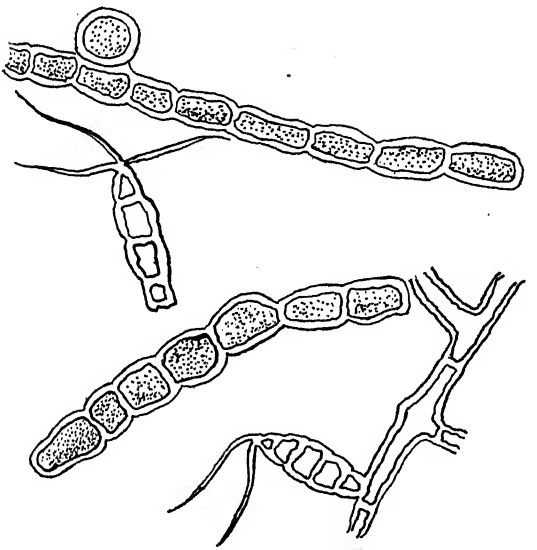
(*c*) *Sulphaguanidine*.—Marked differences were observed at different concentrations. At 0·025% the mycelium and the spores were similar to those on the Control medium but the colour of the spores was old gold. When the concentration was increased to 0·05% the general condition was not much modified except for the presence of few closely septate hyphæ of maize yellow colour. At 0·075% the entire mycelium was closely septate and had warm buff colour. The spores, however, were old gold in colour

with irregular margin and they appeared degenerated (*Vide* Text-Fi . 2). An increase to 0·1% produced hyaline mycelium which appeared as if it was



TEXT-FIG. 2. Shows closely septate hypha and spores with irregular margin of *P. malorum.*

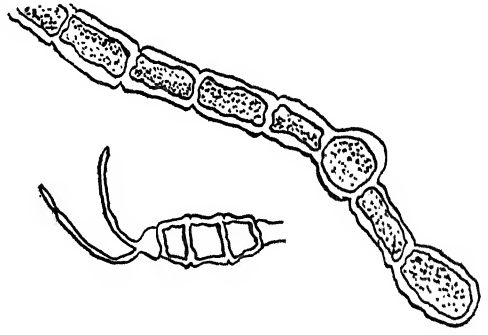
bleached. Chlamydospores of orange buff colour were present in long chains. The number of spores was much reduced. Their margin was, however, less irregular as compared to the medium containing 0·075% of sulphaguanidine (*Vide* Text-Fig. 3).



TEXT-FIG. 3. Shows hyaline mycelium, hyaline spores and chlamydospores.

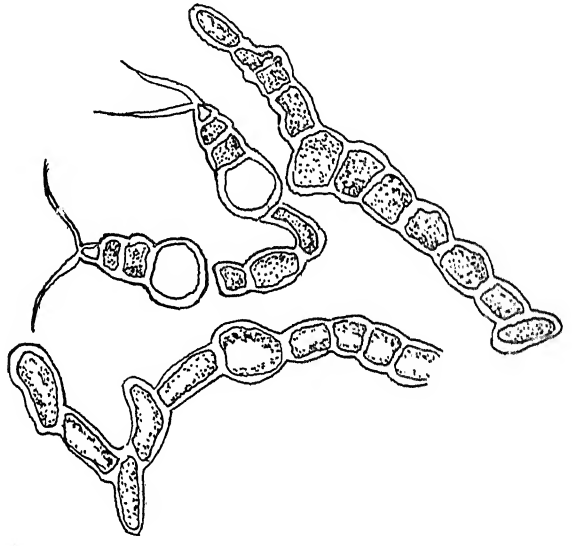
(*d*) *Sulphamerazine.*—Here, again, it was noticed that higher concentrations of the drug induced more marked changes. At 0·025% and 0·05% the mycelium and spores were of the normal type. The colour of

the spores was dark olive buff. They were mostly 4-septate and the number of cilia varied from 2 to 3. At 0·075% and 0·1% most of the spores were old gold in colour but some hyaline ones were also observed. Chlamydospores of antimony yellow colour were present in very long chains (*Vide* Text-Fig. 4).



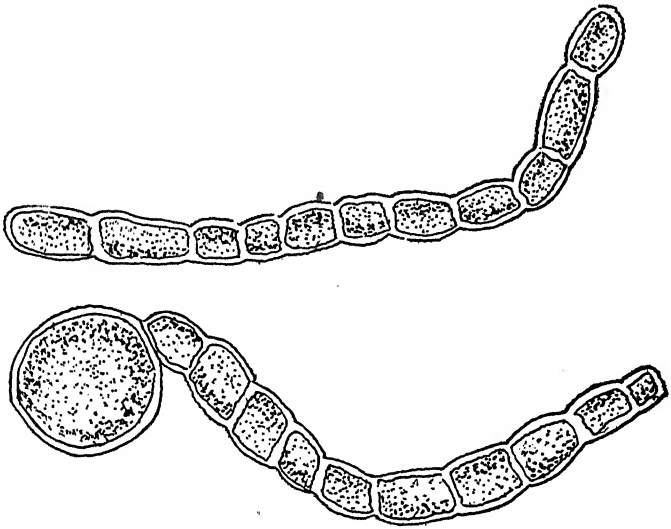
TEXT-FIG. 4. Shows chlamydospores and hyaline spores of *P. malorum*.

(*e*) *Sulphanilamide*.—The mycelium was closely septate, hyaline or orange buff in colour. Chl mydospores were either hyaline or antique brown in colour. Their number and the length of their chain increased with the increase in the concentration of the drug. Only few spores were present. The normal ones were mars yellow in colour but abnormal spores with posterior cell swollen predominated. The number of cilia varied from 2 to 3 (*Vide* Text-Fig. 5).



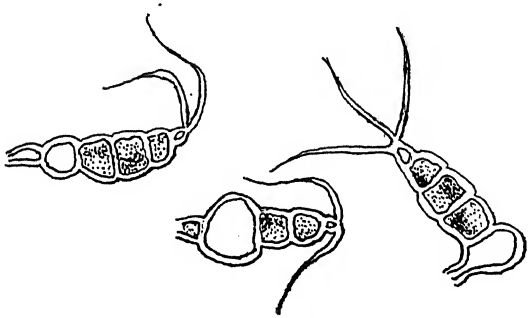
TEXT-FIG. 5. Shows chlamydospores and spores with swollen posterior cell of *P. malorum*.

(*f*) *Sulphapyridine.*—At 0·025% and 0·05% the mycelium and spores were similar to those on the Control medium except for slight differences in the colour of spores. At 0·075% the spores were amber yellow in colour. Numerous chlamydospores of raw sienna colour were produced in chains. The thickness of the wall of chlamydospores increased when the concentration of the drug was increased to 0·1% (*Vide* Text-Fig. 6).



TEXT-FIG. 6. Shows thick walled chlamydospores of *P. malorum.*

(*g*) *Sulphathiazole.*—At 0·025% and 0·05% the mycelium and most of the spores were similar to those on the control medium except for a few spores which had bulbous hyaline posterior cell. The spores were amber in colour and mostly 4-septate. The number of cilia varied from 1 to 3 (*Vide* Text-Fig. 7). The number of spores decreased at 0·075% and a few



TEXT-FIG. 7. Shows bulbous hyaline posterior in spores of *P. malorum.*

degenerated spores were also present. The culture became sterile when the concentration was increased to 0·1%.

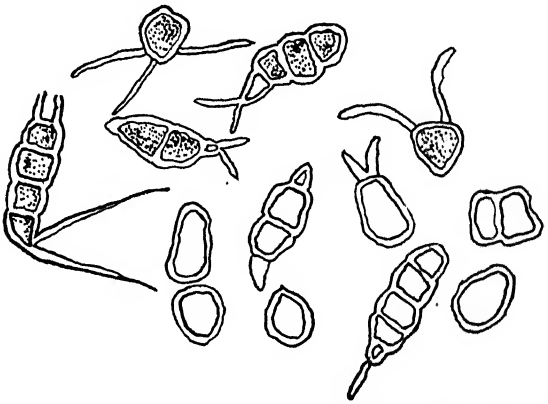
B. *Characters of* P. psidii

(*a*) *Medium A* (Used as Control).—The mycelium was branched, septate, hyaline and filled with homogeneous protoplasm. Spores were spindle-shaped with double-walled septa. They were 4-septate, deep colonial buff in colour and the cilia varied from 1 to 4.

(*b*) *Sulphadiazine*.—Mycelium and spores were similar to those on the control medium except for the colour of the spores which was old gold. Chlamydospores were absent.

(*c*) *Sulphaguanidine*.—General condition on all the concentrations was similar to that on the control medium except for the presence of a few closely septate hyphæ of baryta yellow colour at 0·1% concentration of the drug.

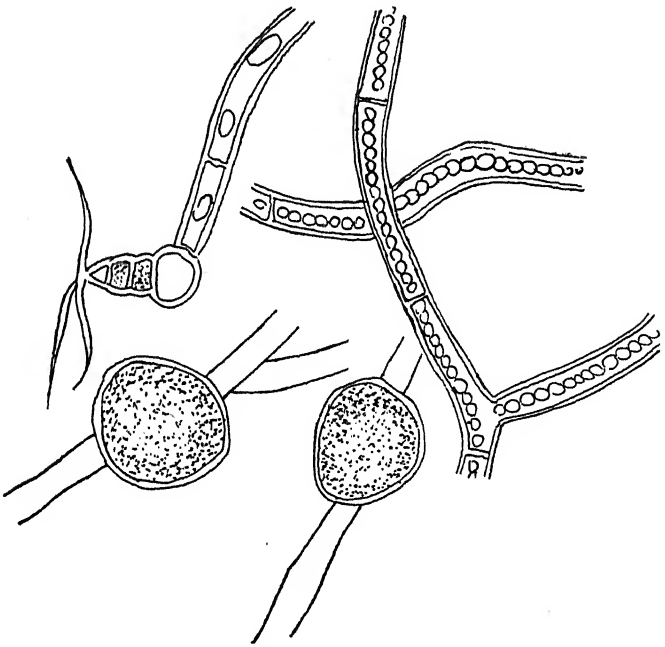
(*d*) *Sulphamerazine*.—At 0·025% and 0·05% the mycelium and the spore were of the normal type. The colour of the spores was buff yellow and the number of cilia varied from 2 to 3. At 0·075% the mycelium was orange buff in colour. Besides normal type of spores, numerous single celled hyaline spores were developed. Their margin was irregular and the cilia were either rudimentary or absent (*Vide* Text-Fig. 8).



TEXT-FIG. 8. Shows aberrant spores with rudimentary cilia of *P. psidii*.

(*e*) *Sulphanilamide*.—The mycelium was broader, hyaline, septate and generally filled with chains of vacuoles. The chlamydospores were of amber colour and were developed in chains. Aniline yellow spores of normal type were observed. They germinated to produce mycelium which was filled

with vacuoles lying far apart and not in chains. The number of cilia varied from 2 to 3 (*Vide* Text-Fig. 9).

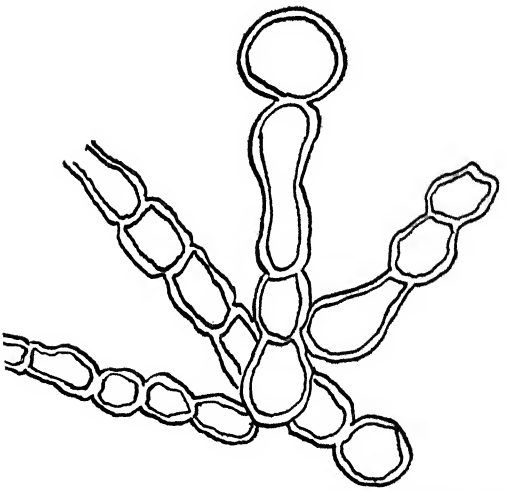


TEXT-FIG. 9. Shows chlamydospores and hyaline mycelium with air vacuoles in *P. psidii.*

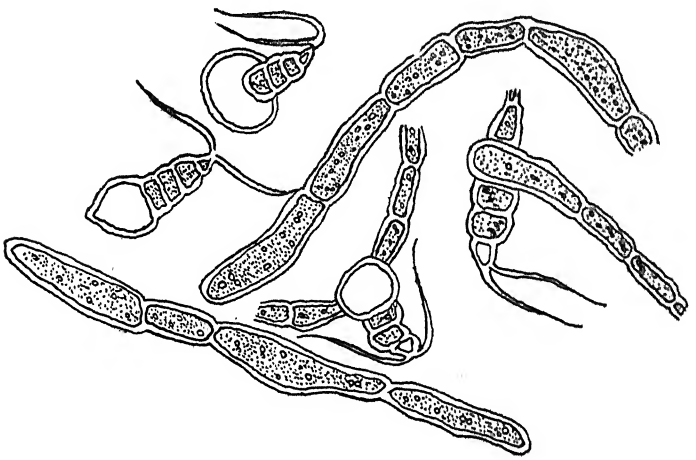
(*f*) *Sulphapyridine.*—At 0·025%, 0·05% or 0·075% the mycelium and spores were similar to those on the Control medium except for slight difference in their colour. The number of cilia however varied from 2 to 3. But at 0·1% long chains of raw sienna-coloured chlamydospores were produced (*Vide* Text-Fig. 10).

(*g*) *Sulphathiazole.*—At 0·025% the mycelium and most of the spores were of the normal type. Some abnormal spores showing a marked swelling of the posterior hyaline cell were however present. At higher concentrations 0·05% to 0·1% there was no difference in the characters of the spores but the mycelium showed the presence of tiny vacuoles (*Vide* Text-Fig. 11).

The microscopic study revealed that there was no marked difference between the control and *P. psidii* on sulphadiazine. Differences at the lower concentrations of some of the other drugs specially with *P. psidii* were also not very marked but in general the addition of Sulpha-drug induced close septation and development of chlamydospores. The number of cilia were also found to vary on different media. Occasionally the spores were ill-formed and in some cases they had even degenerated. These differences



TEXT-FIG. 10. Shows long chains of chlamydospores in *P. psidii.*



TEXT-FIG. 11. Shows mycelium filled with tiny air vacuoles and spores with swollen posterior cell in *P. psidii.*

were particularly pronounced with higher concentrations of the drugs. The formation of a large number of chlamydospores on certain media clearly indicated that they were unsuitable for the normal growth and development of the organisms.

DISCUSSION

From the present investigation it appears that the action of some Sulphadrugs on *Pestalotia* spp. was quite harmful. The presence of sulphanilamide, sulphathiazole and sulphapyridine even in such low concentrations as 0·025% considerably inhibited the growth of both the species of *Pestalotia.*

Such a fungi-static action of sulphonamides was first noticed and reported by Fourneau *et al.*, in 1936. They observed that addition of sulphanilamide to the culture medium greatly delayed the growth of *Aspergillus niger*. Cutting and Gebhardt (1941) while studying the effect of sulphanilamide, sulphathiazole and sulphadiazine on *Actinomyces hominis* found that sulphanilamide at a concentration of 50 or 100 mg. per cent. checked the growth of this organism completely. According to them both sulphathiazole and sulphadiazine were even more effective than sulphanilamide. In the present investigation also sulphathiazole acted more or less in a similar manner and it was practically as injurious as sulphanilamide. Sulphadiazine, however, behaved in a curious manner. No doubt it considerably inhibited the development of *P. malorum* but at lower concentrations it even accelerated the growth of *P. psidii* though higher concentrations did not appear so favourable because a decrease in weight of the fungus resulted with an increase in the concentration of sulphadiazine. Such a detrimental response to an increased concentration of the drug was observed in every case. Noojin and Callaway (1943), working with *Blastomyces dermatitidis* reported that sulphadiazine and Sulphanilamide were most detrimental out of all the seven Sulpha-drugs tested by them. The present investigation, however, indicates that *P. psidii* is not adversely affected by sulphadiazine.

No demonstrable effect on the growth of *Monilia albicans* was observed by Lewis and Hopper (1941), who studied the action of sulphanilamide, sodium sulphapyridine, sulphathiazole, sodium sulphathiazole, sulphadiazine and sodium sulphadiazine upon *Trichophyton gypseum* and *Monilia albicans*. With *Trichophyton gypseum*, however, complete retardation of growth was accomplished by means of sulphanilamide at a dilution of 1%. These results were later confirmed and extended by Dimond and Thompson (1942), who worked with *Trichophyton gypseum* and *Trichophyton purpureum*. They further observed that the action of Sulpha-drugs was fungi-static rather than fungicidal in nature. In the present work also subsequent inoculations of the fungus on Oat Meal Agar medium or on Medium A after their removal from Sulpha-drug gave normal growth. Thus, it was confirmed, that wherever *Pestalotia* spp. showed a poor development it was only due to the Sulphadrug exerting a fungi-static action which became more pronounced with increased concentrations. The fungus was not killed in any case.

In the present investigation the other Sulpha-drugs, *i.e.*, sulphamerazine and sulphaguanidine did not appear to be severe fungi-static agents. In fact, both supported fair amount of growth of the fungus. Senturia and Wolf (1945), working on *Aspergillus fumigatus, Aspergillus niger, A. glaucus, A. sydowi* and *Mucor corymbifer* obtained similar results with both of them

as well as with sulphathiazole and sulphadiazine, provided the quantity of the drug approximated 20 to 30 mg. per culture. Sulphanilamide, however, markedly inhibited the growth of all the organisms used by them. Later Wolf (1946), studying the action of Sulpha-drugs on fungi confirmed the above results. He further noticed that sulphanilamide was fungi-static to most of the organisms used by him. Thus it is evident, that all the previous workers are almost unanimous about great fungi-static properties of sulphanilamide and many have similar views for sulphathiazole also. The extremely poor response of *Pestalotia* spp. towards these compounds is, therefore, not surprising. The differential response of *Pestalotia* spp. with other Sulpha-drugs of the series is probably due to the chemical nature of the drug and the capability of the fungus to react to it. Other observers had also found that the same drug may have different action on different fungi and it is, therefore, not surprising that *P. malorum* and *P. psidii* reacted differently when they were grown on sulphadiazine.

The microscopic study revealed that addition of some Sulpha-drugs brought about morphological changes in the structure of the organisms. The development of abnormal mycelium of *P. psidii* on sulphathiazole and sulphanilamide and abnormal spores of *P. malorum* on sulphadiazine and sulphanilamide and of *P. psidii* on sulphanilamide and sulphathiazole as well as degeneration of spores of *P. malorum* on sulphaguanidine or the formation of hyaline or bleached spores of *P. psidii* on sulphamerazine clearly indicated that the effect of these drugs was not only limited to the growth of the fungus. The morphological characters were definitely modified, possibly on account of the adverse effect of these substances. The development of close septa and the formation of chlamydospores further confirmed that the organisms were unable to carry on their normal activities in the presence of higher concentrations of Sulpha-drugs. It must, however, be admitted that the fungus was not killed by the concentrations used in the present investigation and thus one is unable to use them for a complete control of the disease.

SUMMARY

The effect of various Sulpha-drugs on *Pestalotia malorum* and *Pestalotia psidii* isolated from rotten apples and mummified guavas respectively was studied. Fungi-static action of sulphanilamide and sulphathiazole was quite pronounced. Sulphapyridine also supported only feeble growth, but fair amount of growth was observed on sulphamerazine and sulphaguanidine. Excellent growth of *P. psidii* was observed on sulphadiazine and that was even better than on the control, but with *P. malorum* its fungi-static action was quite pronounced.

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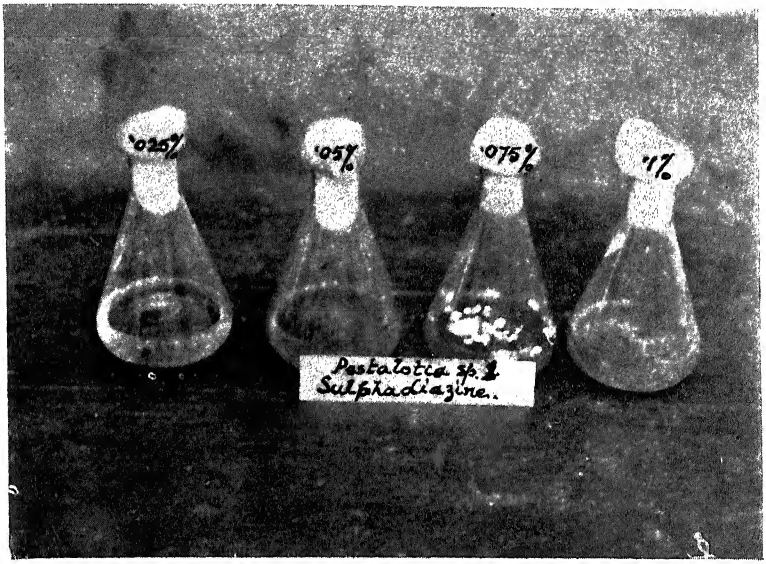

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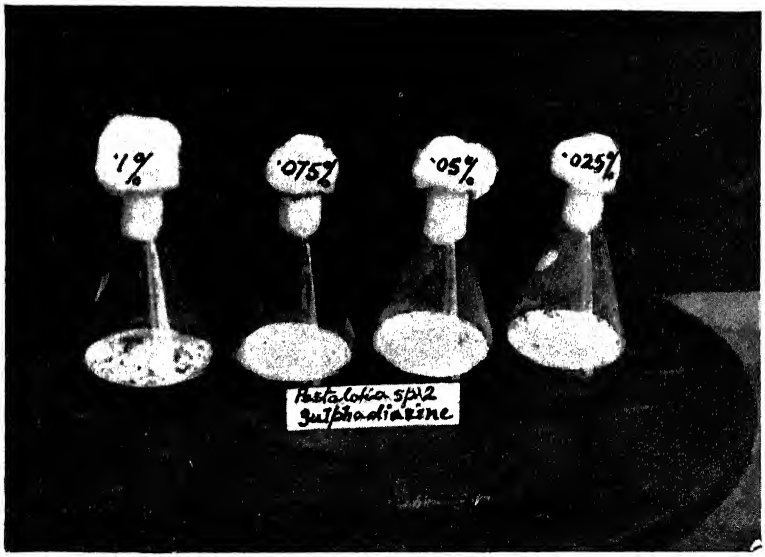

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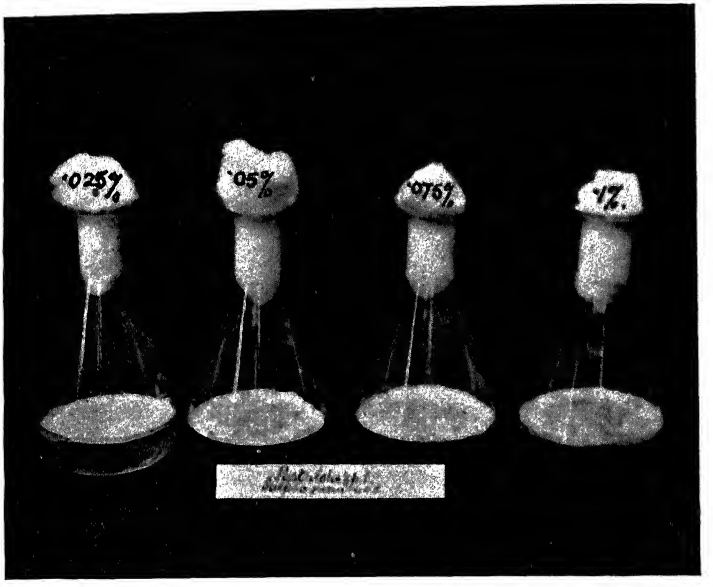

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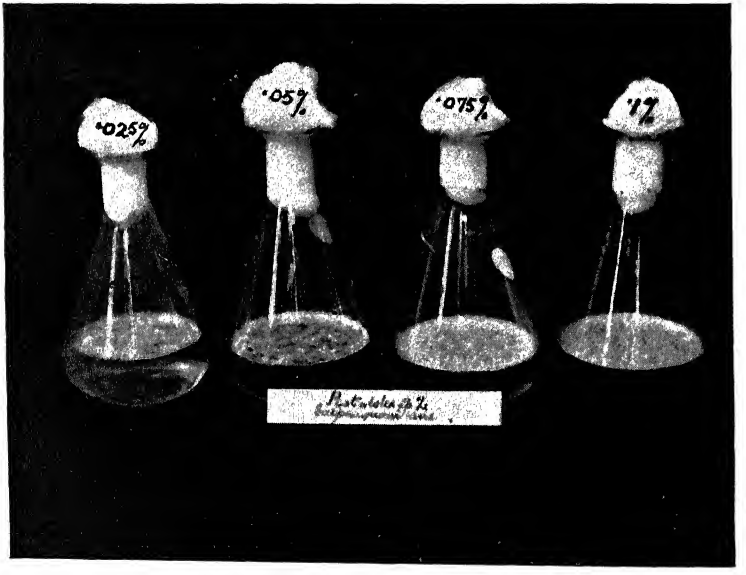

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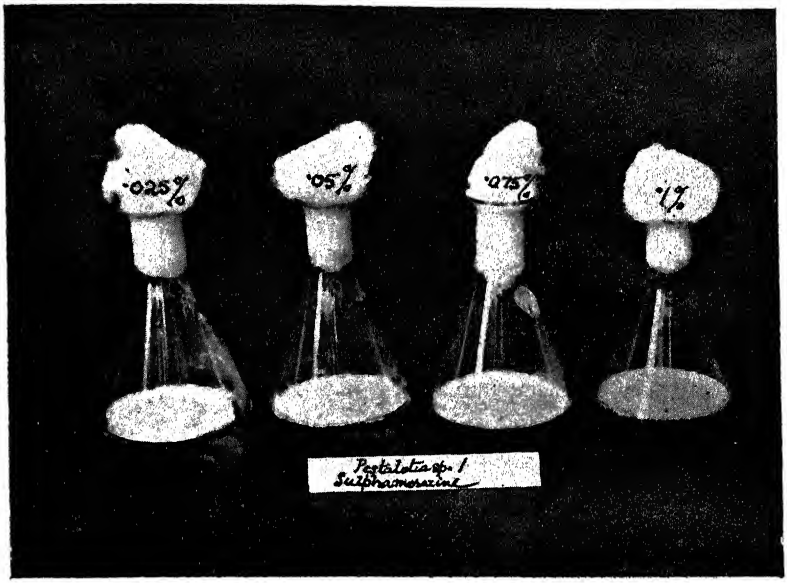

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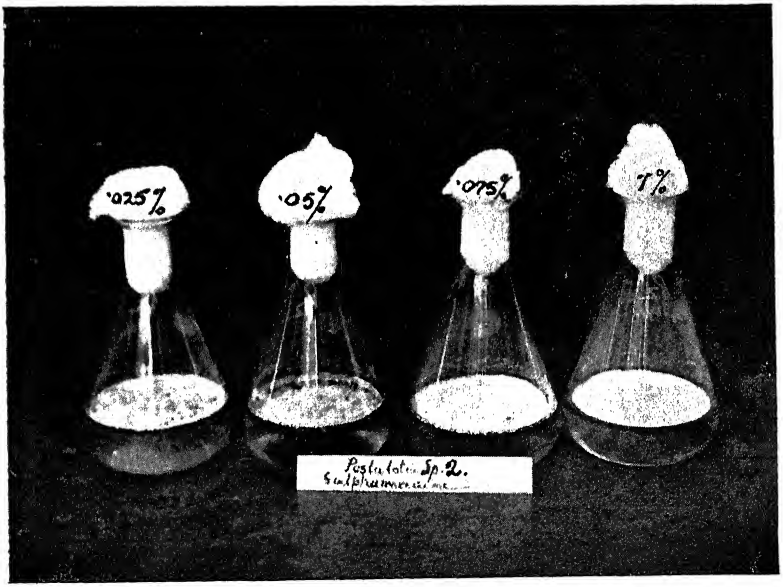

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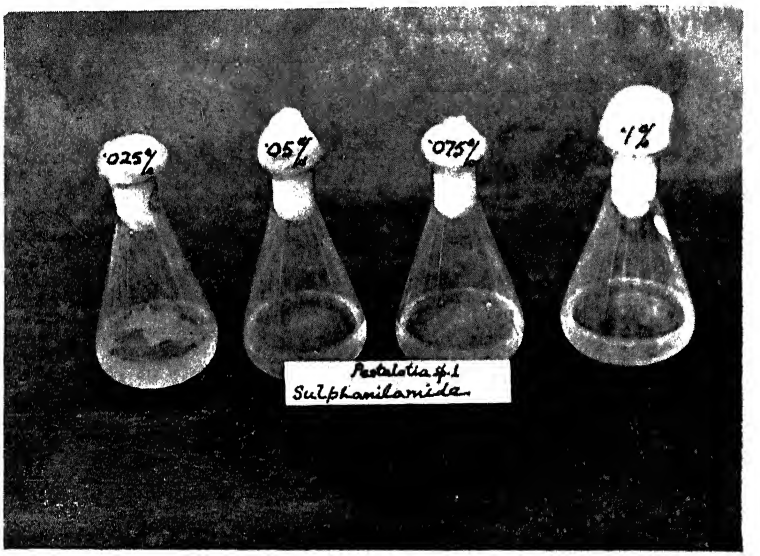

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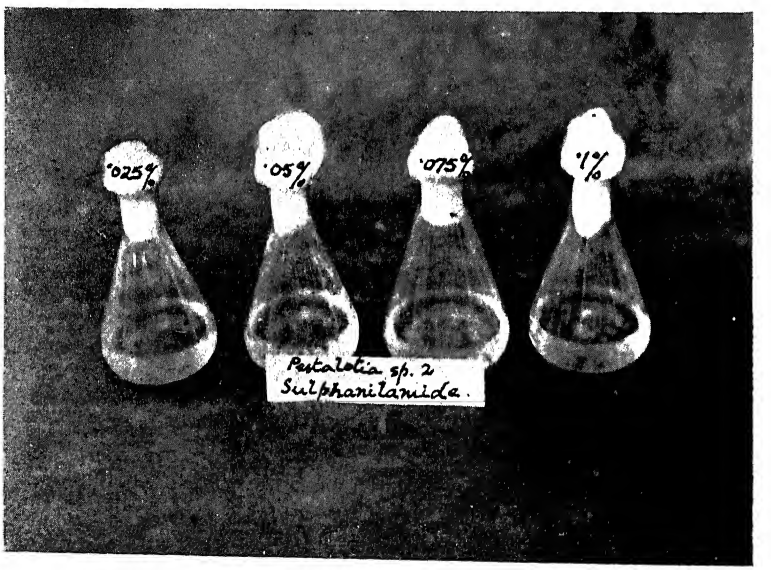

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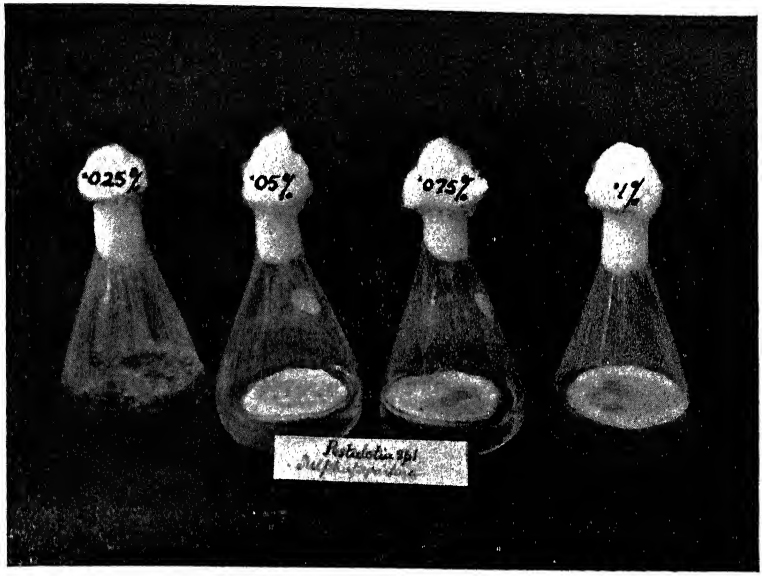

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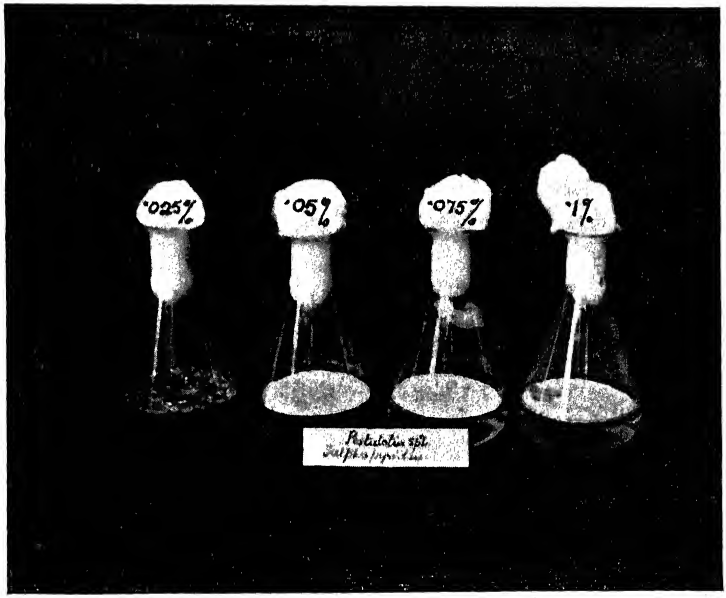

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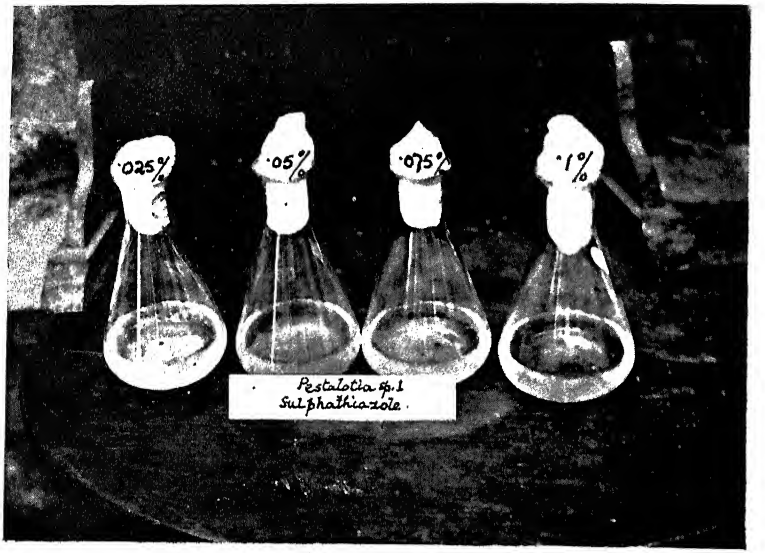

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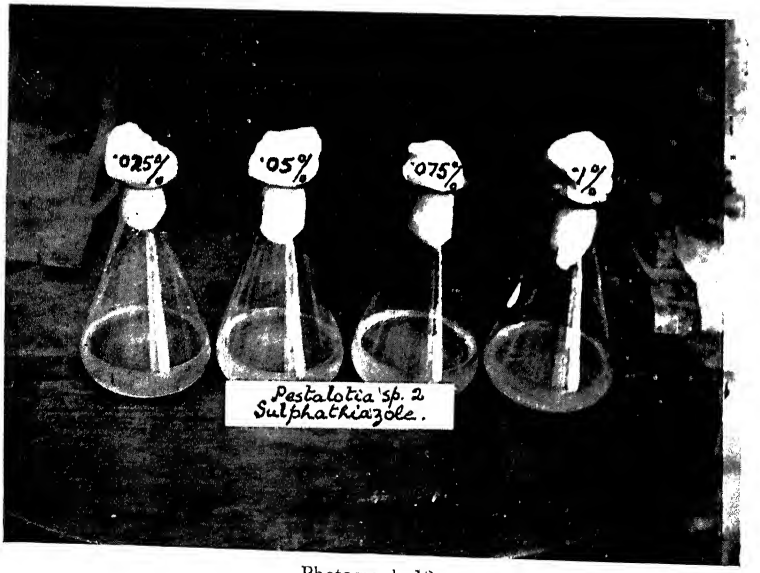

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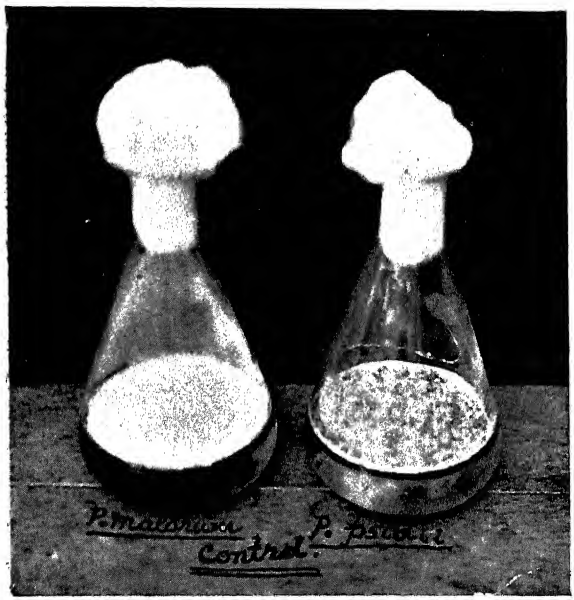

Photograph 13

Microscopic examination revealed that some of the Sulpha-drugs were capable of causing morphological abnormality in mycelium and spores of both the organisms. Production of long chains of chlamydospores of different colours were a common feature wherever poor growth was observed.

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THE STRUCTURE OF CHROMOSOMES WITH SPECIAL REFERENCE TO CHROMOMERES, MATRIX AND LAMP-BRUSH FIBRES*

By M. D. L. Srivastava

(Department of Zoology, University of Allahabad)

The structure of chromosomes is of considerable importance to biologists who accept the chromosome theory of heredity, for these threads carry the genes, the particles responsible for the causation of the phenomenon of heredity. But even to those who are not interested in the character of the specific hereditary substance, the chromosomes must be of significance on account of their role in cellular physiology and embryonic development.

At the very outset mention must be made of two radically divergent views regarding the chromosome structure. One group of older observers held the view that the chromosome is optically heterogeneous, consisting of a series of particles strung together—the chromomeres. This was the view, for example, of Balbiani (1876), Pfitzner (1882), Strasburger (1882, 1884). Another group of contemporary observers contended that the chromomeric granules are resolvable into turns of the chromonematic spiral—the chromosome being a homogeneous chromonema or chromonemata. This was the opinion, for example, of Baranetsky (1880). Strasburger (1884) did not accept Baranetsky's explanation regarding the nature of the chromomeric granules of *Tradescantia*, but numerous subsequent workers down to the present day have claimed to have observed the chromonematic spiral—Schneider (1910), Lee (1921), Kaufmann (1926), Swanson (1943). Chromomeres not resolvable into helices of a spiral have also been claimed to be observed by numerous observers including Darlington (1937), White (1945), Koltzoff (1938), Geitler (1938), Huskins (1942), Straub (1943), etc. The chromomeric interpretation of the chromosomes is held to be strongly supported by the observations on the giant-chromosomes of the salivary gland cells of dipterous larvæ, especially of *Drosophila*, made by numerous investigators, from the time of Balbiani, Kostoff, 1930; Heitz and Bauer, 1933; Painter, 1939; Geitler, 1934; Bauer, 1935; Koller, 1935; Bridges, 1935; Metz, 1935.

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* This paper was read in the Symposium on "The Structure of Chromosomes with Special Reference to Chromomeres" held during the Annual Session of the Academy, 1953.

It may be said at once, however, that the upholders of the chromomeric concept accept the spiralization and despiralization cycle of chromosome, which rests on a secure foundation of numerous careful and accurate observations. Further, it may also be added that at the present time no observer accepts any and every discrete thickening along the chromonematic thread as the chromomere. This term is at present considered applicable only to the granules occurring on the leptotene threads—the true, or Belling's (1928) 'ultimate chromomere'. But in meiosis the prophase chromosome structure has been observed to be a spiral by good many observers—like Newton (1927), Taylor (1931), Shinke (1934), Kuwada and Nakamura (*vide* Kuwada, 1939) and several others, and the leptotene chromomeres have been claimed to be resolvable into helices of a spiral. Koshy (1934), for example, found that the leptotene thread is 'composed of two chromonemata twisted on each other' and that the granular appearance of the chromosome is due to this fact. Similarly, Smith (1932) interpreted the leptotene chromomeres of *Galtonia* as twists in the chromonema. Similar are the conclusions of Naithani (1937) in *Hyacinthus*, Swanson (1943) in *Tradescantia*. The spiral character of the chromomere is upheld by Ris (1945) on the basis of the study of the chromosomes of several grasshoppers like *Chorthippus curtipennis*, *Chorthophaga viridifasciata*, *Melanoplus*, *Hippiscus*, etc. He claims to have succeeded in resolving the chromomeres into turns of a narrowly pitched coil. Further, he claimed that the entire leptotene thread stains uniformly with Feulgen and that there are no 'interchromomeric fibrils'. The chromomeres of zygotene and pachytene are interpreted on a similar basis. Ris could not observe the chromomeres in the diplotene chromonemata and this he considers further evidence against the existence of chromomeres at leptotene stage; for why should they disappear subsequently? Makino and Momma (1950) too have observed that in the spermatocytes of *Podisma* the leptotene thread is uniformly coiled and the so-called 'chromomeres are in reality gyres, or twists, in the chromonemata'.

Despite this weighty evidence against the existence of chromomeres it does not appear to me justifiable to reject the heterogeneous character of the leptotene thread. Contrary to the findings of Ris (1945) and Makino and Momma (1950) that the leptotene thread stains uniformly I have observed that in grasshoppers like *Chrotogonus*, *Acrida* and *Aeolopus* the deeply stained particles or chromomeres are separated by faintly staining fibrilis. This is also evident from the sketches of zygotene—pachytene threads of several animals and plants as given by a number of investigators, *e.g.*, Belling in Lily, Wenrich in a grasshopper, Huskins and Smith in *Trillium* and Darlington in *Fritillaria*. If one accepts the chromomeres as mere helices

of a spiral, it becomes necessary to postulate that along the chromonema zones of spiralization alternate with zones of non-spiralization—an assumption, which seems hardly warranted at all. Besides, it must be remembered that the chromomeres have fixed positions and sizes, as is shown by Wenrich in the chromosomes of *Phrynotettix*. This is especially convincingly seen in the giant chromosomes of the salivary gland cells of dipterous larvæ, especially *Drosophila*. The particulate character of pachytene chromosome has been demonstrated in mouse by Slizynski (1949), where a pattern, bearing a rough resemblance to the dipterous salivary gland chromosome, has been observed. As for the bipartite nature of the leptotene thread, to which Ris (1945) makes reference, it may be pointed out that this is not satisfactorily established. Kaufmann (1931) and Koshy (1934) do claim to have observed that the leptotene thread is double, but most observers take it to be single. Huskins and Smith (1935) indeed do contend that the meiotic metaphase chromosome is octapartite—which implies that the leptotene thread is double, though it may not be directly observable to be such. Nebel even believes it to be quadripartite. But in most animals and plants leptotene thread seems to be single. This, it must be conceded, however, does not materially affect the controversy regarding the nature of the chromomere, for even a single-strand chromonema may be spiralized in such a way as to give the effect of beads strung on a thread.

Chromomeres have been observed in living cells by Belar 1928, Lewis and Robertson (1916), and Chambers (1924). But the same line of argument is advanced against these observations. Ris accepts the genetical longitudinal differentiation of chromosomes, but this, according to him is, expressed in 'differential coiling and not a sequence of discrete bodies'. Differential coiling itself, however, cannot be related to genes, and so the genetical longitudinal differentiation must be relegated to the submicroscopic level. This too need not be considered as an objection against the spiral concept of chromomere. Mention must be made in this connection of Goldschmidt's theory (1940) that the entire chromosome is a unit, and that the effects of gene mutation are produced by intrachromosomal structural changes, the genes themselves having no real existence. This view is hardly acceptable, for it is difficult to see how such changes could have furnished the material basis for macroevolution, but even then one is not forced to accept the chromomeres as the loci for genes, or even identical with them. The chromomere concept is accepted cytologically because these particles are not convincingly demonstrated to be chromonematic twists, and because it is difficult to understand why such fixed spiralized zones should alternate with non-spiralized zones.

The situation is summarized by Kuwada (1939) as follows:—"The chromosome is of the spiral structure, but the spiral thread or the chromonema may be of the chromomeric structure (Fujii, 1931, Fujii and Yasin, 1935; Heitz, 1935). The spiral theory is, therefore, expected to develop into a neo-chromomere theory. It should perhaps, however, be emphasized here that the granular structure of the chromonemata which we actually observe may not represent the ultimate structure of the chromosome".

It is true that Ellenhorn, Prokofyeva and Muller (1935) have succeeded in dissociating optically, by means of ultraviolet rays, the thick discs of the salivary gland chromosomes of the *Drosophila* larva into thinner bands, nevertheless, it is surprising that even in such enormously elongated bands the chromomeres have not been found to be twists in chromonemata. Ris (1945) claims to have done it, but on the whole it may be safely considered as not established.

The chromonema has been reported by several investigators to be embedded, to a varying degree, in a ground substance, or matrix. Fujii (1926) (*vide* Kuwada, 1939) reported that in the pollen mother cells of *Tradescantia reflexa* the gaps between the turns of the spiralized chromonemata are filled with matrix or hyalonema.

Fujii regarded it as a protective colloid, preventing chromonemata from coming in contact with each other. Kuwada (1939) holds that in both mitosis and meiosis the single-coiled or double-coiled structure of the chromonemata is determined by the peculiarity of the matrix. Ris (1945), however, challenges the existence of a ground substance. He writes that "probably many a matrix in the literature is nothing but the apparent connection between faintly staining outer loops, running at an even distance from the darker core of the chromosome where the chromonemata overlap". Makino's (1936) photomicrographs of the matrix in diakinetic and metaphase chromosomes in the grasshopper *Podisma* is explained away on this basis. Nevertheless, the presence of a matrix has been quite convincingly established. Smith's (1932) diagrams of somatic anaphase chromosomes of *Galtonia* show the chromonemata to be embedded in a matrix. The chromonema is definitely double-stranded and the coiling is probably of the orthospiral type causing relational coiling. But that this matrix can be taken to be identical with the overlapping outer loops is definitely ruled out. I have observed a similar relationship of matrix and chromonemata in the second telophase of chromosome of *Chrotogonus* in fixed and stained material. It is difficult to decide in this case whether the chromonema is a two-strand structure. In all probability it is a single-strand element, but even if it be

accepted that the chronema is double-stranded, the two strands are not at all drawn apart and the question of overlapping of outer loops does not arise at all. In *Chrotogonus* the second anaphase chromosome also shows the differentiation of chromonema and matrix in aceto-carmine-treated cells. Further, Yuasa (1953) has recently studied the action of potassium salts on the matrix and chromonema. In *Aeolopus* I have observed that the matrix and the chromonemata of second metaphase chromosomes are differentially stained in acetocarmine, especially after treatment with potassium ferricyanide. Both the matrix and the chromonemata may be damaged, but there is no chance of confusing one with the other. So the view that the chromonemata are naked throughout the chromosome cycle and their loops may give the impression of the presence of a matrix may be safely dismissed.

The same criticism has been labelled against what I consider to be a derivation of the matrix, namely, lamp-brush fibres'. Lamp-brush chromosomes were first described in the enormously grown yolky eggs of Sauropsida, fishes, amphibians, insects. These are enormously elongated, diffuse diplotene-diakinetic chromosomes, in which the deconcentration and despiralization of chromosomes proceeds to an enormous extent. The chromosomes come to possess numerous lateral processes taking on a lamp-brush appearance (Koltzoff, 1938; Duryee, 1938). Now lamp-brush processes have been described in diplotene-diakinetic bivalents in spermatocytes in certain insects as well. These have been reported to exist in the pachytene and diplotene bivalents in certain grasshoppers by Hsu (1948), Ris (1945), Srivastava (1950), and are shown in the drawings of Hearne and Huskins and certain earlier observers (*vide* Wilson, 1928). Duryee (1937, 1938, 1939 and 1941), who made a very thorough study of these processes in amphibian eggs, where the diplotene stage may persist for two years or so, came to the conclusion that these arise as lateral processes from the chromomeres and are shed off before the maturation divisions occur. More recently (1950) Duryee has reaffirmed his earlier conclusions. Koltzoff (1938) also regards them as processes arising from chromomeres.

Another explanation of these fibres has also been put forth. According to Ris (1945) these are simply the outer gyres of chromonemata. Serra (1947) gives a similar explanation, the hairs, according to him, being the gyres of chromonemata and rod-like nucleoplasm deposited on them. [Swanson (1943) and Kuwada (1939) do not describe lamp-brush fibres as such, but refer to the fuzzy appearance of diplotene bivalents and ascribe it to the minimum or minor spiral.] This view is not supported by observations made by me on *Chrotogonus* and *Aeolopus* and by Hsu on *Phlæoba infumata* and *Catantops humilis*, in which cases the lamp-brush fibres are

already present in the pachytene stage and do not become more numerous and larger at the diplotene and the diakinetic stage as they should on this explanation. At diplotene they are equally clearly observable in bivalents in which the four strands are separated. In this case Ris's explanation of the main body being more deeply stained than the processes holds good only if it is assumed that the bivalent is octapartite (as Huskins holds) and not quadripartite, which does not seem to be the case in the grasshopper. The disparity in intensity of staining is so great that one must assume the lamp-brush fibre to be different from the chromonemata. Besides, even at diakinesis they are wide apart, which would mean that spiralization is loose even at this stage.

Painter (1940) considered lamp-brush processes to be bundles of chromosomes resulting from endomitosis. Hsu (1948) has put forth a tentative hypothesis linking up spiralization, heterochromatization, and lamp-brush fibre formation. He writes "where the intra-cellular conditions favour the exhibition by the X of a lamp-brush structure and cause the autosomes to be compactly coiled (as in the early spermatogonial divisions) negative heteropycnosis of the X will result. Where the conditions are reversed, as in the meiotic prophase, the X chromosome will remain compactly coiled, while the autosomes all show the typical lamp-brush structure; under the circumstances the X may be described as positively heteropycnotic." This is in consonance with Goldschmidt's views. But it must be urged that spiralization and lamp-brush fibres occur at the same time during diakinesis—this is not to be expected on Hsu's hypothesis.

It seems, therefore, that the lamp-brush fibres are independent of chromonemata and are derived from the matrix—as suggested by Painter in 1941. I am also of the view that the chromatic threads connecting the bivalents reported in numerous Hemiptera (Wilson, 1928) and damselflies by Srivastava and Das (1953) are also of this nature. They are considered as expression of heterochromatization, but that means only that the matrix of the heterochromatin is physico-chemically different from that of the euchromatin. It is true that many investigators have shown heterochromatic chromosomes to be tightly coiled at a time when the autosomes are uncoiled, or poorly coiled (Shinke, 1937; Coleman, 1940, 1941, 1943; White, 1945; Ris, 1945; Mickey, 1946; Makino and Momma, 1950). This could be expected even without a direct knowledge, but the differential behaviour of euchromatin and heterochromatin during chromosome cycle must result from constitutional differences. Caspersson (1941) actually attributes differential protein frameworks to euchromatin and heterochromatin. Caspersson and Schultz (1938) found heterochromatin especially

active in synthesising nucleic acid. Schrader (1941) thinks the matrix of heterochromatin is adhesive.

I find that adhesion between chromosomes and also connection between bivalents by means of chromatic threads is brought about to a high degree in the spermatocytes of *Aeolopus* on subjecting these insects to cold. This means that the matrix can be affected experimentally in this way.

Examination of lamp-brush chromosomes in fixed eggs of Tortoises has not enabled me to decide the issue quite satisfactorily.

It would seem, therefore, that the chromonema has the capacity not only to attract particles of its own kind from the substrate but also to synthesise a nucleoprotein matrix of a different constitution, which may be drawn out as fibres and subserve protective and nutritive functions.

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ZONA PELLUCIDA IN THE EGGS OF *BOS INDICUS* AND *BOS BUBALUS**

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Introduction

Though the oogonia and the early oocytes are naked in the beginning they gradually develop and get enveloped by follicular epithelium, and the egg proper ultimately gets surrounded by a thin membranous layer, the zona pellucida, which becomes complicated with growth. The origin of egg membranes has long been a subject-matter of controversy. They were studied as early as 1880 by Van Beneden. Detailed descriptions of, more or less, convincing nature are provided by the studies of Gatenby (1922 *b*) and Van der Stricht (1923). In 1937 Aykroyd described the zona pellucida in the egg of woman. According to her the zona makes its first appearance when the follicle is one-layered only and she attributed its formation to the follicle cells. Marie Guthrie and K. B. Jeffers (1938) have studied the zona pellucida of cat's ovum. The present paper is an account of the study of the zona pellucida in the egg of the cow (*Bos indicus*) and the buffalo (*Bos bubalus*).

Material and Technique

The material was obtained from two domestic mammals named above. The animals were slaughtered by severing off the neck, the ovaries were taken out without any loss of time, to check post-mortem changes, and transferred to Ringer's salt solution. They were then cut into bits of suitable size and transferred to required fixatives. Bouin's picro-formol-acetic fluid was found invaluable as a fixative for the study. The chief stain used was Mann's methyl-blue-eosin and some times Ehrlich's hæmatoxylin followed by Mann's methyl-blue-eosin was also used. Among osmic preparations Ludford's and Kolatchev's methods gave very successful results. Centrifuged material fixed in Regaud-Tupa and stained after Champy-Kull displayed clearly the striations of the Zona. Material fixed in acidulated silver nitrate for the study of Vitamin C displayed the structure of theca successfully.

* Abridged from D. Phil. Thesis.

OBSERVATIONS

Histological study of the ovary, based on Bouin's picro-formol-acetic preparation stained in Mann's methyl-blue-eosin, reveals that the ovary consists of a mass of connective tissues, the stroma, into which are scattered large number of specialized more spherical cells, having spherical nuclei usually with conspicuous nucleoli, the ova. The stroma of the ovaries contains, spindle-shaped connective tissue cells, plain muscular fibres and a number of epithelium-like interstitial cells, some of which are derived from the germinal epithelium and appear to be capable of dividing.

A layer of flattened epithelial cells forming the outermost layer of the ovary lying just internal to the peritoneum constitutes the germinal epithelium (Fig. 1). In the underlying stroma, slightly larger and more spherical cells derived from the germinal epithelium are abundant (Fig. 1). These are neatly rounded structures often found in groups of two or four. Each ovum enlarges, becomes more spherical, with a distinct prominent nucleus having usually a conspicuous nucleolus. Further, it becomes surrounded by a layer of epithelial cells, forming a primary follicle. It is interesting to note that the cells forming the follicular layer are flattened and arranged with their long axes parallel to the wall of the oocyte (Fig. 2). The zona has not appeared till this stage.

The follicle grows and becomes two-layered. The elongate follicle cells now show a tendency to become columnar (Fig. 3). With further growth these cells multiply forming a multi-layered follicle. When a multi-layered follicle has been formed a crescentic space appears in the follicular layers towards one side of the ovum. This space is filled with a fluid called liquor folliculi.

Simultaneously with these changes in the follicular epithelium other differentiating features also appear. Important among these are, firstly, the appearance of a homogeneous deeply staining membrane, the zona pellucida, separating the egg and the follicular cells; and secondly, the outer surface of the follicles getting invested by cells of mesenchymatous origin forming the theca. The zona pellucida, in cross-section, is but a thin one-layered cuticle (Fig. 4). It did not show any traces of network on oblique and tangential sections as mentioned by Thing (1918) in turtles.

These differentiations are further pushed to an extreme, resulting in the formation of a mature follicle, the Graafian follicle. The liquor folliculi increases in quantity gradually so much so that in the latter stages it dominates the whole picture. The follicle cells surrounding this liquid filled

space are arranged in two layers: (1) a thinner layer of follicle cells lying next to the theca known as the zona granulosa, and (ii) a little hillock of cells, called cumulus oophorus, lying on one side and carrying the ovum (Fig. 5). The zona pellucida becomes more prominent and the theca becomes differentiated further forming two well-marked layers, the inner one, theca interna, and the outer one, the theca externa (Fig. 5).

In such advanced phases of growth the nuclei of the follicular epithelium lie closely on the zona pellucida which becomes thicker and divided into two concentric layers, the outer layer consisting of clear space and the inner showing faint radial striations (Fig. 6). In its fully formed stage the zona pellucida appears to be made up of three distinct layers (Figs. 7 and 8) (i) the outermost lightly-coloured layer without any striations, (ii) the middle layer which is the most prominent of the three and is distinctly striated transversely—the zona striata, and lastly (iii) the innermost homogeneous layer, quite thin, and lying in between the zona striata and the limiting membrane of the egg. Last layer is added quite late in development. All these stages are quite distinct in Kolatchev's preparations.

Discussion

Earlier workers have described the zona pellucida as a membrane traversed by a great number of fine radial canals and have thus named it zona radiata. About the radial striations, believed to be canaliculi for conducting nourishment, there are different views. Some believe them to be crossing the entire zona, others trace them only in its outer portion, whereas still others believe that the striations are more strongly indicated in the internal part of the zona.

Marshall (1893) described the structure of an ovum that he got from the fallopian tube of swine and also some of common rodents. According to him, a faint concentric layer of hyaline material may be seen in the zona. Absolutely no radial striations were found. The zona is so clear that the details of the enclosed yolk are seen, as if, through clear glass.

Mammalian zona pellucida is believed to be radially striated and traversed by canalicular prolongations of the follicle cells by Van der Stricht (1905). Gatenby (1922) in a well advanced oocyte described the egg membranes to consist of theca externa and interna, a double-layered follicular epithelium, a zona radiata, a cortical layer of fibrillæ and the true cell-wall or limiting membrane. The cell-wall which at an earlier stage is a thin membrane is connected to the zona by a large number of cortical fibrillæ which according to Gatenby, probably, serve the dual purpose of attaching

the zona firmly to the egg and of acting as living protoplasmic connections between the nutrient-supplying follicle and the receptive interior of the egg.

The best description of the structure of a reptilian egg is provided by Bhattacharya and collaborators (1929) as follows: "In a well advanced oocyte, besides the usual theca and a single layered follicular epithelium we find a well developed zona pellucida consisting of a striated region of zona radiata and a homogeneous layer more or less corresponding to that described by Champy in fishes. In silver and osmium preparations the layer seems to be studded with an immense number of black granules which we identify as Golgi bodies. The presence of this immense number of Golgi elements gives to this layer a thicker and darker consistency in contrast to the striated layer of zona radiata which appears to be lighter, in colour. We believe that this dense layer is a part of the zona radiata. It is not present in all stages of development. In earlier stages only the striated region of the zona is present. Underlying this homogeneous layer is a transparent region of more or less radially arranged fibrillar prolongations from the extreme periphery of the egg. This layer, which we call fibillar layer, appears to correspond to the cortical layer of the fibrillæ described by Gatenby in Ornithorhynchus and to the striated layer of the extreme cortical region of the egg in Petromyzon described by Champy."

The question of origin of the zona pellucida still remains controversial in spite of so much work carried out by distinguished workers in various groups of animals. There are three different views about the formation of the egg membranes.

(1) There are some who believe that the zona arises from the cytoplasm of the egg. Van Beneden, 1880, working on bat, supports the view that the zona arises from the secretions of the cytoplasm and puts forth the following argument: In a biovular follicle two eggs may lie side by side without having any follicular cells in between them. Yet the zona is clearly formed at such a place. Such examples are quite frequent and may be difficult to explain, if only the follicular epithelium is supposed to secrete the zona.

(2) Majority of the present-day workers appear to believe that the zona radiata of vertebrates, particularly of mammals, develops from the follicular epithelium alone. Von Ebner (1900), Flemming (1882), Fischer (1905), and Retzius (1889) support this view. Gatenby (1922) thinks that the substance of the zona is formed in direct relationship to the cells of the follicle, and the cytoplasm of the egg, probably, takes merely a secondary or stimulating part in the production of this important membrane. It is interesting to

note that according to Gatenby the zona pellucida commences to form at the time when the follicle changes from the single to the double-layered condition. According to Brambell also, in birds, the differentiation of the two kinds of cells and the assumption of the many-layered condition of the follicle are immediately followed by the formation of zona striata. Besides the above-mentioned authors, Van der Stricht (1905), Thing (1918) and Brambell (1925) are all of the opinion that the zona pellucida is formed by the follicle cells probably by the intracellular substance.

(3) Among those who believe that the zona is formed under the influence of both the follicular epithelium and the cytoplasm of the egg, Marshall (1893) is probably the first. He remarks, "Between the ovum and the inner layer of the follicle cells a thick non-cellular layer with faint radial striations, the zona radiata, is formed apparently as a cuticular secretion from either the ovum or the follicle cells." Gatenby (1922 *b*) believes that there are three possible methods of the development of the zona pellucida in mammals. The zona might develop from the follicular epithelium, it might develop from the egg cytoplasm or it might develop under the influence of both the egg cytoplasm and the follicular epithelium. According to Thing (1918) the zona pellucida is formed by two or three different elements: "(*a*) the fundamental homogeneous substance filling up the spaces between, (*b*) *a* system of numerous canals or tubules which enclose, (*c*) filaments or prolongations of the epithelial cells which are connected with the surface of the yolk."

In the present case the zona pellucida starts appearing, as Gatenby has mentioned in Ornithorhynchus, at the time that the follicular epithelium becomes double-layered. The zona at this stage takes eosin strongly when stained with Mann's methyl-blue eosin after Bouin's fixation method. After Regaud-Tupa fixation and iron-hæmatoxylin stain it becomes deep blue. Later, as the follicular layer grows, zona also grows in size. In a four or five layered follicle the zona is seen quite distinct but unstriated. At this stage its connection with the follicle is cells seen to be more intimate than with the cytoplasm of the egg, as the limiting membrane appears to be separated from the zona by a delicate clear space. This intimate relationship of the follicle cells and the zona evidently proves that the latter is being formed by the follicular secretions. From this stage onward the intimate contact continues till the egg is actually discharged. A couple of layers of follicle cells are even carried by a discharged ovum. On maturity the zona appears to be made up of roughly three layers: (i) an outer zone that appears light brown in colour in Kolatchev's unbleached preparation. This layer does not appear to be striated; (ii) underneath this is the inner broader zone

having faint radial striations, the zona radiata; and (iii) the innermost layer that lies between the striated layer and the limiting membrane of the egg proper. This layer appears homogeneous and is probably secreted by the cytoplasm of the egg. The fibrillæ as described by Prof. Bhattacharya in the tortoise and by Gatenby in Ornithorhynchus are absent.

From the detailed structural study of the egg membranes I am led to follow the view put forward by Gatenby (1922 *b*) that the zona is formed under the influence of both the egg cytoplasm and the follicular epithelium. In the early stage it is secreted by the egg cytoplasm, but as the growth proceeds the responsibility of the secretion of the zona is shared by the cells of the follicular layers.

SUMMARY

The structure of the ovary has been fully described. Smaller oogonia are without follicular epithelium, which develops with further growth. The follicle is surrounded by a theca externa and a theca interna. The zona pellucida appears as a thin cuticular membrane which becomes complicated with growth of the follicle and in a mature egg shows three regions; an outer non-striated layer, a middle radially striated layer and an inner non-fibrillar layer. The zona arises from both the egg cytoplasm and the follicular epithelium, the former secreting it in the early stages and the latter sharing the responsibility in the latter stage.

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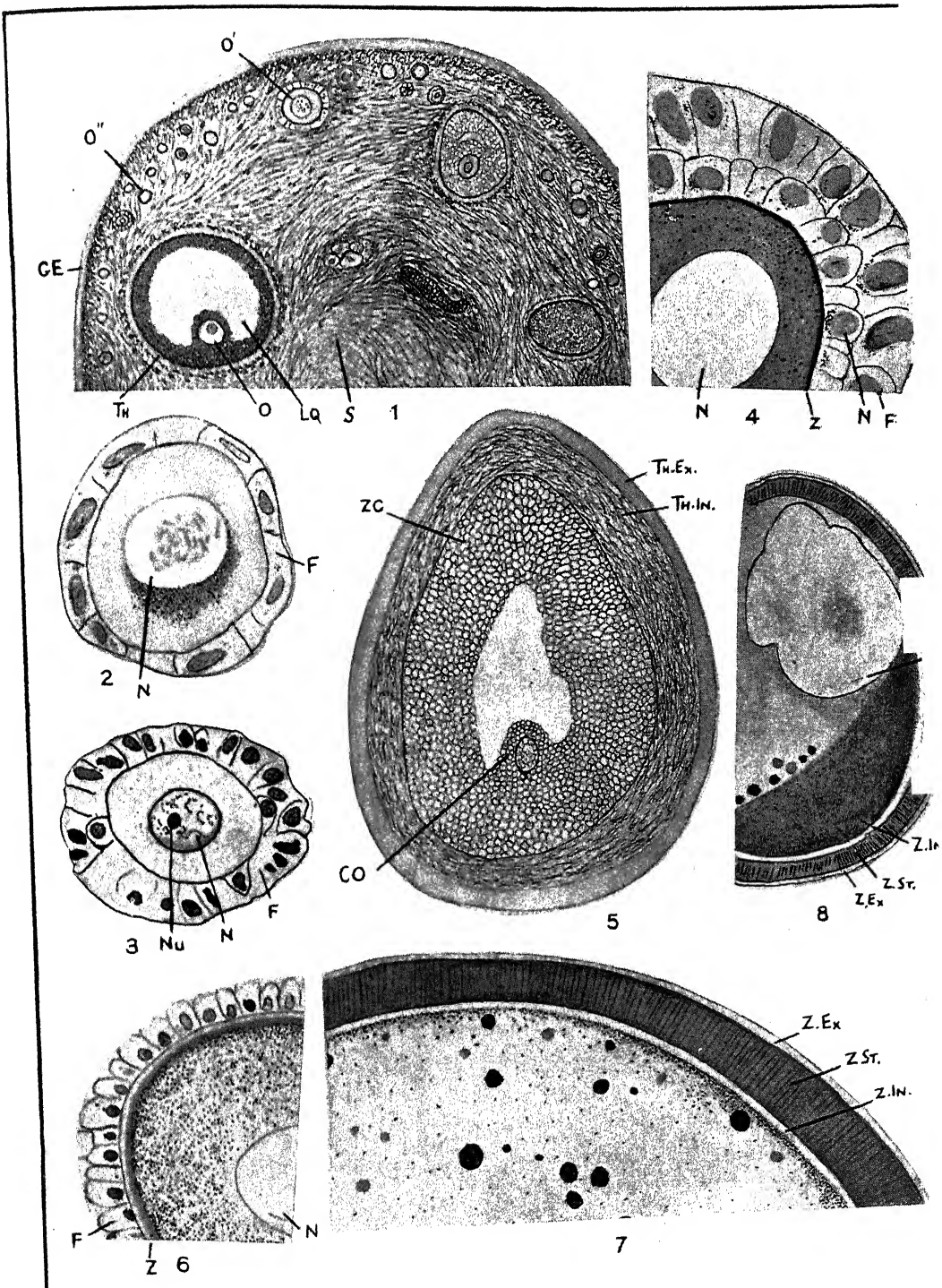
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LETTERING

CO., Cumulus oophorus; *F.*, Follicle cells; *GE.*, Germinal epithelium; *L.*, Liquor folliculi; *N.*, Nucleus; *Nu.*, Nucleolus; *O.*, Ovum with a single-layered follicle; *O'.*, Ovum with a single layered follicle; *O".*, Naked oogonia; *S.*, Stroma; *Th.*, Theca; *Th.ex.*, Theca externa; *Th.in*, Theca interna; *Z*, Zona pellucida; *Z. Ex.*, Outer layer of zona; *Z.G.*, Zona granulosa; *Z.In.*, Inner layer of zona; *Z.St.*, Zona striata.

EXPLANATION OF FIGURES

FIGS. 1-8. Fig. 1. T.S. of the ovary of cow. Bouin' Mann's methyl-blue eosin. Free hand sketch. Fig. 2. An young oocyte surrounded by a single-layered follicle, the cells of which are arranged with their long axes parallel to the wall of the oocyte. Cajal unbleached, 10×90. Fig. 3. An young oocyte the follicle of which is becoming double-layered and the cells of the follicular epithelium show a tendency of becoming columnar. Bouin, Mann's methyl-blue eosin, 10×90. Fig. 4. Two layered follicle with distinct zona pellucida. Ludford, bleached, 10×90. Fig. 5. A follicle surrounded by distinct theca. Acetic-acid-silver nitrate solution, untoaned, 10×40. Fig. 6. Zona pellucida growing into two-layered structure Ludford, bleached, 10×90. Fig. 7. Fully formed, theca layered zona pellucida. Kolatchev, 10×90. Fig. 8. Part of a centrifuged ovum. Regaud-Tupa. Champy Kull, 10×40.

Note.—Figures 2 to 8 have been drawn with the help of camera lucida.